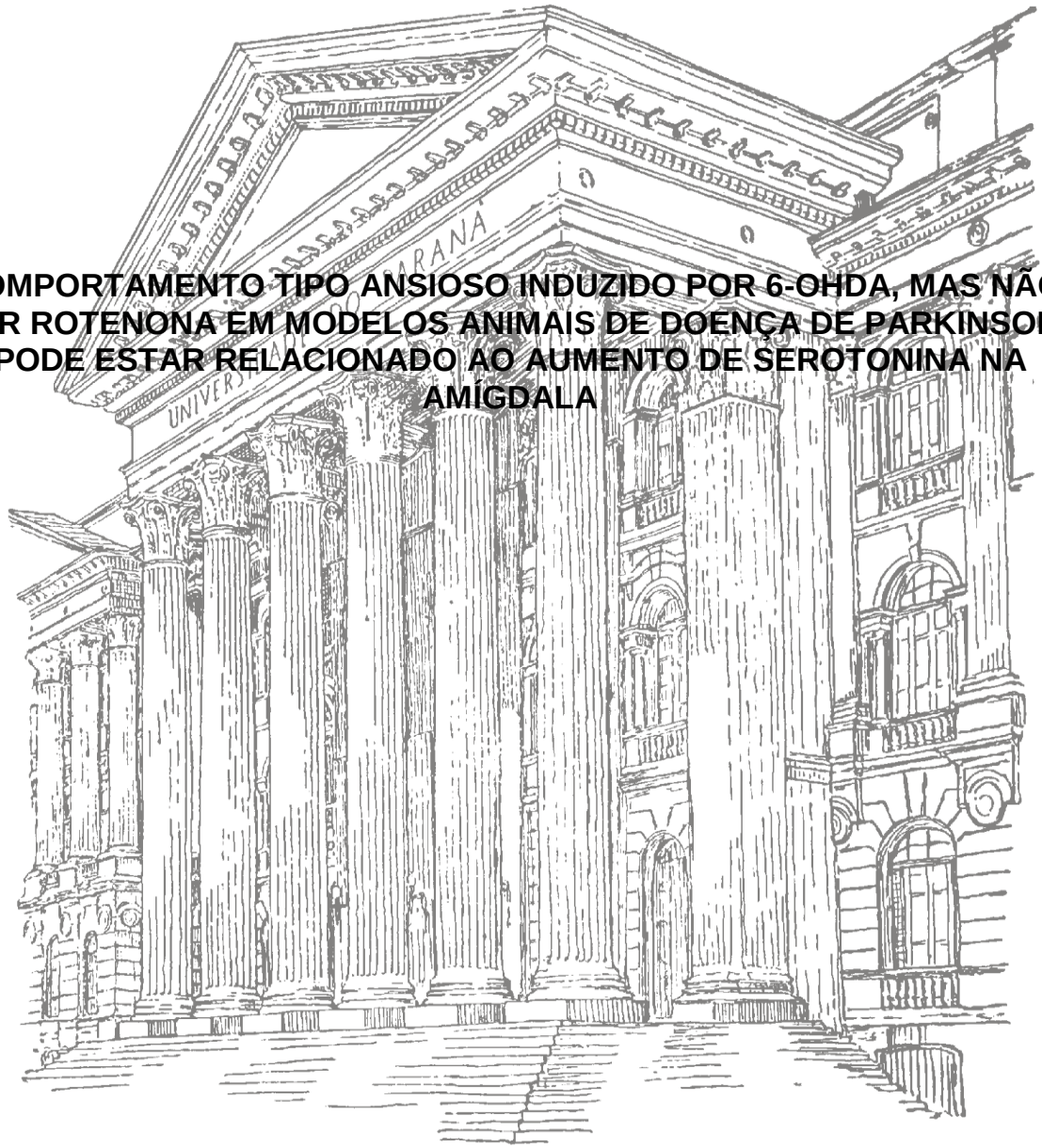
UNIVERSIDADE FEDERAL DO PARANÁ

JEANE CRISTINA FONSECA VIEIRA



COMPORTAMENTO TIPO ANSIOSO INDUZIDO POR 6-OHDA, MAS NÃO POR ROTENONA EM MODELOS ANIMAIS DE DOENÇA DE PARKINSON, PODE ESTAR RELACIONADO AO AUMENTO DE SEROTONINA NA AMÍGDALA

CURITIBA
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Dissertação apresentada ao Curso de Pós-Graduação em Farmacologia, do Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Farmacologia.

Orientadora: Profª. Drª. Maria Aparecida B. F. Vital.

Co-orientadora: Profª. Drª. Janaína M. Zanoveli

CURITIBA
2015

Ministério da Educação
UNIVERSIDADE FEDERAL DO PARANÁ
Setor de Ciências Biológicas
Programa de Pós-Graduação em Farmacologia

PARECER

A Comissão Examinadora da Dissertação de Mestrado intitulada "Comportamento tipo ansioso induzido por 6-OHDA, mas não por rotenona em modelos animais de Doença de Parkinson, pode estar relacionado ao aumento de serotonina na amígdala.", de autoria da pós-graduanda JEANE CRISTINA FONSECA VIEIRA sob orientação da Prof.ª Dr.ª Maria Aparecida Barbato Frazão Vital e composta por: Prof.ª Dr.ª Maria Aparecida Barbato Frazão Vital (Presidente - Farmacologia - UFPR); Prof. Dr. Roberto Andreatini (Farmacologia – UFPR) e Prof. Dr. Silvio Marques Zanata (Patologia Básica – UFPR), reuniu-se e, de acordo com o Regimento Interno do Programa de Pós-Graduação em Farmacologia, a pós-graduanda foi APROVADA. Para a devida publicação o trabalho deverá sofrer as modificações sugeridas, que serão conferidas por sua orientadora. Em Curitiba, 12 de junho de 2015.

Prof.ª Dr.ª Maria Aparecida Barbato Frazão Vital (Presidente - Farmacologia - UFPR)

Prof. Dr. Roberto Andreatini (Farmacologia - UFPR)

Prof. Dr. Silvio Marques Zanata (Patologia Básica – UFPR)

Centro Politécnico – Caixa Postal 19031 – CEP: 81531-990 – Curitiba/PR
Telefones: (41) 3361-1693 – Fax (41) 3266-2042 – pgfarmacologia@ufpr.br
http://farmacologia.bio.ufpr.br/posgraduacao

ATA DO JULGAMENTO DA 108ª DEFESA DE DISSERTAÇÃO DE MESTRADO

Ao décimo segundo dia do mês de junho do ano de dois mil e quinze, às quatorze horas, no Auditório do Departamento de Farmacologia, Anexo I, Setor de Ciências Biológicas da Universidade Federal do Paraná, reuniu-se a Comissão Examinadora da Dissertação de Mestrado de autoria da pós-graduanda **JEANE CRISTINA FONSECA VIEIRA**, intitulada "Comportamento tipo ansioso induzido por 6-OHDA, mas não por rotenona em modelos animais de Doença de Parkinson, pode estar relacionado ao aumento de serotonina na amigdala.", sob orientação da Prof.ª Dr.ª Maria Aparecida Barbato Frazão Vital e composta por: Prof.ª Dr.ª Maria Aparecida Barbato Frazão Vital (Presidente - Farmacologia - UFPR); Prof. Dr. Roberto Andreatini (Farmacologia – UFPR) e Prof. Dr. Silvio Marques Zanata (Patologia Básica – UFPR). A Banca Examinadora iniciou os trabalhos. A candidata teve quarenta e cinco minutos para expor oralmente seu trabalho, sendo em seguida arguida durante trinta minutos por cada um dos membros da Banca e tendo trinta minutos para responder a cada uma das arguições. No final da sessão, a Comissão Examinadora emitiu o seguinte parecer: Aprovada, Para a publicação, o trabalho deverá sofrer as modificações sugeridas, que serão conferidas por sua orientadora. Nada mais havendo a tratar, a Presidente deu por encerrada a sessão, da qual foi lavrada a presente ata, que será assinada pela Presidente e pelos demais Membros da Comissão Examinadora em Curitiba, 12 de junho de 2015.

Prof.ª Dr.ª Maria Aparecida Barbato Frazão Vital (Presidente - Farmacologia - UFPR)

Prof. Dr. Roberto Andreatini (Farmacologia – UFPR)

Prof. Dr. Silvio Marques Zanata (Patologia Básica – UFPR)

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RESUMO

A Doença de Parkinson (DP) é a segunda doença neurodegenerativa mais comum em adultos com mais de 65 anos. Os sinais e sintomas característicos da DP são classicamente motores e resultado principalmente de uma degeneração particularmente progressiva de neurônios dopaminérgicos na substância negra parte compacta (SNpc) e uma consequente perda de dopamina no corpo estriado. A DP também é caracterizada por sintomas não motores como sintomas psiquiátricos que muitas vezes incluem transtornos de depressão e / ou ansiedade. A ansiedade em DP pode representar uma reação psicológica para o desenvolvimento de outros sintomas durante a progressão da doença, mas existe evidência de que um aumento de distúrbios de ansiedade pode estar relacionado com alterações neuroquímicas na DP. Neste sentido, e tendo em conta a necessidade de mais estudos envolvendo métodos de investigação que apresentem ansiedade em modelos animais de DP, este estudo foi desenvolvido. No nosso protocolo experimental, os animais receberam a neurotoxina 6-OHDA na substância negra através de cirurgia estereotáxica bilateralmente ou rotenona intraperitoneal (ip) durante 10 dias. Para avaliar o comportamento do motor, todos os animais foram avaliados no teste do campo aberto após 24 horas e 21 dias de tais exposições. Os testes do labirinto em cruz elevado (LCE) e o condicionamento ao medo contextual (CMC) foram usados para avaliar os comportamentos de ansiedade após 21 dias de procedimentos dos grupos *sham* e 6-OHDA ou veículo (óleo de girassol) e rotenona. Após os testes comportamentais, foram coletados desses animais: estriado, córtex pré-frontal e amígdala dissecados para futura análise de monoaminas por HPLC. Um dia após a cirurgia, ou no final do tratamento ip, observou-se que os animais lesionados mostraram hipolocomoção e menor freqüência de elevação no teste de campo aberto, os quais foram espontaneamente revertidos na última avaliação motora (dia 21). No último dia do experimento, o modelo de 6-OHDA mostrou comportamento tipo ansiogênico no teste de LCE e CMC, uma redução de dopamina (DA) e noradrenalina (NA) em todas as estruturas coletadas. Curiosamente, um aumento de serotonina (5-HT) na amígdala foi observado. Para rotenona, nossos dados não revelam comportamento tipo ansiedade, nem alterações neuroquímicas dos níveis de 5-HT na amígdala. Estes resultados indicaram que a serotonina pode exercer uma importante influência em sintomas de ansiedade em DP, além da modificação das outras monoaminas.

Palavras-chave: Doença de Parkinson. Ansiedade. Serotonina. 6-OHDA. Rotenona.

ABSTRACT

Parkinson's disease (PD) is the second most common neurodegenerative disease in adults over 65 years. The characteristic signs and symptoms of PD are classically motors and resulted primarily from a particularly progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and a consequent loss of dopamine in the striatum. PD is also characterized by non-motor symptoms as psychiatric symptoms often include disorders of depression and / or anxiety. The anxiety in PD may represent a psychological reaction to the development of other symptoms during disease progression, but there is evidence that an increase in anxiety disorders may be related to neurochemical changes in PD. Therefore, and taking into account the need for further research studies of methods experience anxiety PD animal models, this study was designed. In our experimental protocol, the animals received the neurotoxin 6-OHDA in the substantia nigra bilaterally through stereotactic surgery or rotenone intraperitoneally (ip) for 10 days. To evaluate the behavior of the engine, all animals were evaluated in the open field test after 24 hours and 21 days of such exposure. The elevated plus maze (EPM) and the contextual fear conditioning (CFC) were used to evaluate the anxiety behaviors after 21 days following the sham and 6-OHDA or vehicle (sunflower oil) and rotenone. After the behavioral testing, animals were those listed: striatum, prefrontal cortex and amygdala dissected for future analysis by HPLC monoamines. One day after surgery, or at the end of ip treatment, it was observed that the lesioned animals showed hypolocomotion and lower high frequency in the open field test, which was spontaneously reversed in the last motor assessment (21). On the last day of the experiment, the 6-OHDA model showed anxiogenic-like behavior in the EPM and CFC test, a reduction of dopamine (DA) and noradrenaline (NA) in all the collected structures. Interestingly, an increase of serotonin (5-HT) was observed in the amygdala. For rotenone, our data reveal no behavior like anxiety or neurochemical changes in 5-HT levels in the amygdala. These results indicated that serotonin may play an important influence on anxiety symptoms in PD, as well as modification of other monoamines.

Key-words: Parkinson’s Disease. Anxiety. Serotonin. 6-OHDA. Rotenone.

LISTA DE ABREVIATURAS

5-HT - Serotonina

6-OHDA - 6-hidroxidopamina

CMC – Condicionamento ao medo contextual

DA - Dopamina

DHPG - Dihidroxifenilglicol

DOPAC - Ácido 3,4-dihidroxifenilacético

DP - Doença de Parkinson

HVA - Ácido homovanílico

HPLC – Cromatografia líquida de alta eficiência

LCE – Labirinto em cruz elevado

MAO-B - Monoamino Oxidase-B

mAch – Receptores muscarínicos de acetilcolina

MPTP - fenil-1,2,3,6-tetrahidropiridina

ROS – Espécies reativas de oxigênio

SNC – Sistema nervoso central

SN – Substância negra

SNpc - Substância Negra parte compacta

LISTA DE FIGURAS

SUMÁRIO

1 INTRODUÇÃO

1.1 DOENÇA DE PARKINSON

A Doença de Parkinson é a segunda desordem neurodegenerativa mais comum em adultos com mais de 65 anos a nível mundial, ficando atrás somente da Doença de Alzheimer (LAU e BRETELER, 2006; PHANI, LOIKE e PRZEDBORSKI, 2012). Considerada classicamente como uma desordem de movimento, a DP idiopática possui um diagnóstico baseado na presença de um conjunto de sinais e sintomas motores como rigidez, bradicinesia, tremor em repouso e distúrbio reflexo postural (PREDIGER *et al*., 2012). Pacientes com DP também tipicamente desenvolvem uma postura de parada e podem perder reflexos posturais normais, levando a quedas e algumas vezes ao confinamento a uma cadeira de rodas (DAUER e PRZEDBORSKI, 2003)

O aparecimento do prejuízo motor está relacionado com a neurodegeneração de aproximadamente 50% dos neurônios dopaminérgicos do mesencéfalo e a perda de 80 a 90% do conteúdo de dopamina (DA) estriatal (FUCHS *et al.,* 2004; WARRAICH *et al*., 2009).

Entretanto, uma cuidadosa observação dos pacientes descritos por James Parkinson há 200 anos, revela a existência de sintomas não motores na DP (TAYLOR *et al*.,2009). Estes sintomas não motores incluem disfunção autonômica (gastrointestinal e disfunção cardiovascular com hipotensão ortostática), deficits olfativos, distúrbios do sono e / ou neuropsicológicos (cognitivos e demência) e sintomas psiquiátricos (depressão e ansiedade). Há evidências de que eles podem até serem anteriores aos sinais motores no início precoce da doença (DUNNET e LINDGREN, 2012).

1.1.1 Etiologia

A causa da DP ainda é desconhecida, mas postula-se que diversos fatores estejam relacionados à gênese da doença, incluindo alterações genéticas, fatores ambientais, excitotoxicidade, neuroinflamação e estresse oxidativo (PRZEDBORSKI, 2005). Além do estresse oxidativo e consequente disfunção mitocondrial, estudos

demonstram que a inflamação é outro importante mecanismo envolvido no desenvolvimento e progressão da DP (PIEPER *et al.*, 2008; REALE, *et al*., 2009).

Pacientes com parkinsonismo familiar representam cerca de 10% a 15% dos casos. Alterações genéticas que afetam genes como os que codificam a α-sinucleína, a parkina e componentes do sistema proteossômico foram encontrados em alguns casos de parkinsonismo (HERNÁNDEZ-MONTIEL, 2006; LEE and LIU, 2008).

Também há uma hipótese em que a DP seria desencadeada por um agente neurotrópico até então desconhecido, que poderia ser um vírus, o qual inicialmente afeta o intestino e o sistema olfatório, causando agregação da α –sinucleína. Posteriomente, o agente neurotrópico se propagaria através do SNC dando origem aos Corpúsculos de Lewy no trajeto e, após muitos anos, atingiria a substância negra (HAWKES, TREDICI e BRAAK,2007).

1.1.2 Fisiopatologia

Uma importante característica fisiopatológica encontrada em encéfalos *post mortem* de pacientes com DP é a presença de corpúsculos de Lewy em diversas regiões mesencefálicas. Esses corpúsculos são caracterizados pela presença de agregados protéicos compostos por proteínas como: parkina, ubiquitina e principalmente pela proteína sináptica α-sinucleína (DAUER e PRZEDBORSKI, 2003; CICCHETTI *et al*., 2009; KIM *et al*., 2014). Vários mecanismos pelo qual maiores níveis de α -sinucleína poderia causar diretamente ou estar associada a toxicidade neuronal cerebral foram propostos incluindo a ruptura da membrana, interferência com as vias de sinalização, alterando o tráfico da vesícula entre outros (CAVINESS, 2014).

Uma mal formação de α –sinucleína também propicia características tóxicas, sendo um evento resultante de outros como mutações genéticas, peroxidação lipídica, diminuição do pH, presença de íons metálicos e estresse do retículo endoplasmático (LEE *et al.*, 2011; KIM *et al.*, 2014).

Braak e colaboradores (2004) levantaram a hipótese de que corpúsculos de Lewy apareceriam a princípio no sistema nervoso entérico e depois se propagariam para o cérebro, sugerindo um transporte retrógrado ativo de α-sinucleína. Estudos recentes indicam que a α-sinucleína é ativamente transportada do instestino para o

núcleo dorsal motor do nervo vago (HOLMQVIST *et al*., 2014). Além disso, foi visto que, corpúsculos de Lewy são transferidos a partir de uma célula para outra, não só em células cultivadas in vitro, mas também em cérebros de modelos animais de DP (DESPLATS *et al*., 2011; HANSEN *et al*., 2011)

O progresso da DP evoluiria de estágios pré-sintomáticos até a disseminação dessas inclusões proteicas pela substância negra e componentes dos sistemas autonômico, límbico e somatomotor (Braak *et al.,* 2004). Como a DP caracteriza-se especialmente de um déficit motor, sua base orgânica é a degeneração das aferências da SN ao estriado. Essas aferências utilizam a dopamina que normalmente facilitaria a alça motora direta ativando células no putâmen. Em essência, a depleção de dopamina fecha o funil que alimenta a atividade na Área Motora Suplementar via núcleos da base e VLo (afererência para núcleo ventrolateral) (BEAR *et al.*, 2008). Porém, o rompimento das interações entre os diversos sistemas de neurotransmissores, têm consequências na sintomatologia da DP. A função motora do estriado é dependente do equilíbrio alcançado entre dopamina e acetilcolina, enquanto que a atividade dopaminérgica dentro da SNpc é modulada por si e inervações glutamatérgica e GABAérgica. Além disso, neurônios serotonérgicos podem também afetar a libertação de dopamina no estriado na DP e interações serotonina-dopamina são fortemente implicadas em comportamentos relacionados com a recompensa, como a dependência e em distúrbios neuropsiquiátricos (BARONE, 2010). A noradrenalina presente no locus coeruleus encontra-se depletada na DP antes mesmo da depleção de dopamina na SN, com estudos mostrando o envolvimento da via do locus coeruleus, em instabilidade postural, fenômeno *on-off* e comportamento de congelamento (*freezing*) (DELAVILLE, DEURWAERDÈRE e BENAZZOUZ, 2011).

1.1.3 Tratamento farmacológico

Até o momento, os tratamentos farmacológicos e não farmacológicos disponíveis oferecem alívio sintomático, mas a doença permanece incurável. A natureza progressiva da DP e suas manifestações clínicas (motoras e não motoras), associadas a efeitos colaterais precoces e tardios da intervenção terapêutica, tornam o tratamento da doença bastante complexo.

As estratégias terapêuticas atuais têm o objetivo de reduzir a severidade dos sintomas com o uso de quatro categorias principais de drogas: (1) anticolinérgicos, (2) agonistas de receptores dopaminérgicos, (3) inibidores da MAO, (4) L-dihidroxifenilalanina (L-DOPA). A L-DOPA, droga de frequente escolha para o tratamento de DP, uma vez no SNC é convertida em dopamina pela enzima aminoácido aromático descarboxilase (AADC). Muitas vezes é necessária a combinação de fármacos de diferentes classes para melhor controle dos sintomas. Embora as medicações dopaminérgicas serem amplamente usadas no tratamento da DP, ainda há necessidades clínicas não satisfeitas, incluindo falta de drogas adequadas para tratar a neurodegeneração, complicações motoras ou psicoses associadas com tratamento crônico com L-DOPA, e sintomas não motores (deficiências cognitivas e transtornos de humor). Além disso, sob tratamento a longo termo, a eficácia da L-DOPA flutua e pode causar sérios efeitos colaterais. Novos medicamentos efetivos não só para sintomas motores, mas também para os não motores são requisitados (SHIMIZU e OHNO, 2013).

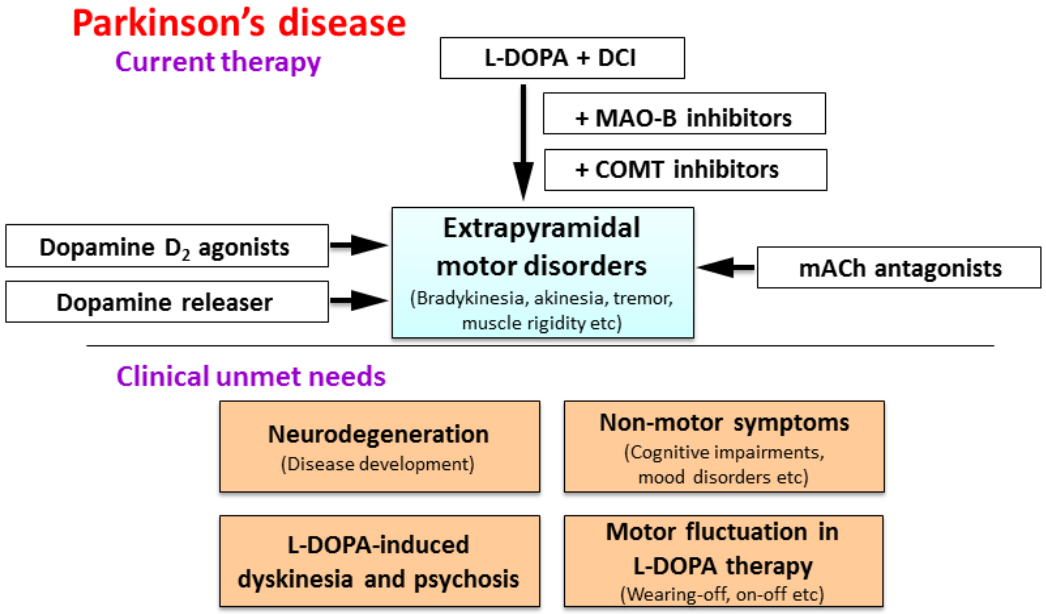

FIGURA 1. Terapêutica atual e necessidades clínicas no tratamento da DP. Adaptado de: SHIMIZU e OHNO (2013).

1.2 DOENÇA DE PARKINSON E ANSIEDADE

1.2.1 Prevalência de ansiedade na DP

Além das dificuldades motoras encontradas na DP, a ansiedade presente também afeta a qualidade de vida sendo portanto um transtorno importante a ser compreendido. Transtornos de ansiedade complicam o diagnóstico clínico e tratamento da DP, já que estudos analisando ansiedade nessa patologia são limitados (DISSANAYAKA *et al*., 2010).

Taxas de ansiedade em populações com DP excedem aquelas encontradas em populações normais e afetadas por doença crônica (BOGDANOVA E CRONIN-GOLOMB, 2012; PICILLO *et al.,* 2013) e tem sido postulada como um fator de risco para DP, uma vez que, como depressão, pode se manifestar antes do início dos sintomas motores (REICHMANN *et al.,* 2009).

O transtorno do pânico, transtorno de ansiedade generalizado e fobia social são os transtornos de ansiedade mais comuns relatados. Ansiedade e transtornos depressivos muitas vezes coexistem na DP e pode preceder sintomas motores (DISSANAYAKA *et al*., 2010). Fobia social que é relatada como comum em pacientes parkinsonianos, está associada a uma supressão sustentada de ambas transmissão dopaminérgica e de atividade nos receptores de DA (SCHNEIER *et al.*, 2000;. STEIN *et al.*, 2002).

A prevalência de ansiedade em DP é díspar, com estudos relatando taxas de 5% (LAUTERBACH e DUVOISIN, 1991) a 69% (KULISEVSKY *et al*., 2008). Cerca de 30% de pacientes que sofrem de depressão na DP também experimentou desordem de pânico, e um adicional de 11% expressa ansiedade generalizada, em comparação com 5,5% da população geral (NUTI *et al*., 2004).

1.2.2 Neurobiologia da ansiedade na DP

O início da ansiedade na DP pode preceder o aparecimento da sintomas motores ou pode se desenvolver após o diagnóstico. A ansiedade que começa alguns anos antes do início de sinais motores pode ser uma manifestação "pré-motora" de DP relacionada a alterações neurobiológicas precoces. Já a ansiedade

que ocorre após o diagnóstico da DP poderia apresentar uma causa diferente dessa anterior (DISSANAYAKA *et al.,*2014).

As alterações presentes na ansiedade além de uma relação ao quadro psíquico também teriam uma origem neuroquímica, estando associadas com um estado de excessiva excitabilidade do Sistema Nervoso Central (SNC) e/ou prejuízo dos sistemas GABAérgico, noradrenérgico e o serotonérgico (TABACH, 2011). De acordo com os estágios patológicos da DP sugeridos por Braak, a perda de células serotonérgicas nos núcleos da rafe é evidente antes da degeneração dopaminérgica nigroestriatal (BRAAK *et al*., 2004).

O sistema serotonérgico desempenha um papel crucial no controle de vários processos fisiológicos, incluindo as funções psico-emocional, sensório-motor, cognitivo e funções autônomas. Neurônios de 5-HT estão localizados no núcleo da rafe e envia axônios para várias regiões do cérebro, incluindo o córtex cerebral, áreas límbicas, gânglios basais, diencéfalo e da medula espinhal (SHIMIZU e OHNO, 2013). Notavelmente, MENZA *et al.* (1999) descobriram que os pacientes com DP que carregava o alelo curto do transportador 5-HT pontuavam significativamente acima do que os não-portadores em escalas de ansiedade. Isto sugere que os fatores genéticos envolvendo esses receptores podem desempenhar um papel na patogênese da ansiedade na DP (PREDIGER *et al*., 2012).

O teor de 5-HT e a densidade de transportadores de 5-HT em regiões do prosencéfalo (por ex. estriado) são reduzidos em pacientes com DP. Alternativamente, os receptores pós-sinápticos de 5-HT_{1A} e 5-HT_{2A} são desrregulados em resposta ao déficit funcional de neurônios 5-HT. Várias linhas de evidências revelaram que os receptores 5-HT_{1A} desempenham um papel importante no controle de funções motoras e melhora várias desordens extrapiramidais tais como sintomas parkinsonianos motores, e deficiência motora induzida por L-DOPA. Além disso, receptores 5-HT_{1A} estão implicados na patogênese e tratamento de distúrbios de humor e de ansiedade, bem como de deficiências cognitivas que são vistas frequentemente em pacientes com DP (AKIMOVA, LANZENBERGER e KASPER, 2009; SHIMIZU e OHNO, 2013).

1.2.3 Terapêutica atual

Resultados controversos foram obtidos com drogas que atuam como Inibidor Seletivo de Recaptação de Serotonina (ISRS). Enquanto estudos conduzidos com humanos mostraram que o ISRS fluoxetina pode reduzir discinesia provocada pelo agonista de receptor dopaminérgico apomorfina outros mostraram que o ISRS citalopram poderia aumentar a bradicinesia (NICHOLSON e BROTCHIE, 2002). Não se conhece a real causa dessas diferenças observadas.

Os antidepressivos que aumentam a disponibilidade de serotonina, como os ISRS, induzem seus efeitos sobre a ansiedade e a depressão clínica após um tratamento prolongado, geralmente após 4 semanas de uso contínuo. Estudos mostram que o efeito terapêutico ocorre por alterações neuroplásticas em áreas encefálicas envolvidas com respostas emocionais. Estudos conduzidos utilizando análise neuroquímica, bem como registro eletrofisiológico mostram que o uso prolongados de ISRS aumentam o conteúdo extracelular de serotonina em algumas áreas, além de induzir alterações como hiper ou hiporregulação de receptores serotonérgicas em áreas encefálicas específicas (para revisão, ver GRAEFF e ZANGROSSI, 2010). Essas alterações, além de outras, estão envolvidas no efeito terapêutico dessas drogas.

Quando há ativação de autoreceptores 5-HT_{1A} pela serotonina ou agonistas exógenos observa-se hiperpolarização neuronal e redução do disparo neuronal (CELADA, BORTOLOZZI e ARTIGAS, 2013). Porém, o autoreceptor 5-HT_{1A} sofre uma dessensibilização ao longo de 2-3 semanas de uso contínuo do ISRS, permitindo assim um aumento do disparo neuronal com consequente aumento na concentração extracelular de serotonina em diferentes áreas encefálicas (BLIER, 2010; CELADA, BORTOLOZZI e ARTIGAS, 2013).

É provável que as ações ansiolíticas e antidepressivas de agonistas 5-HT_{1A} sejam mediadas por ambos os receptores pós-sináptico e pré-sináptico (Figura 2). Receptores 5-HT_{1A} estão principalmente localizados nas regiões límbicas (hipocampo e na amígdala) e septo lateral, que estão implicadas na geração de distúrbios de humor e de ansiedade. Sugere-se que os agonistas 5-HT_{1A} produzam efeitos ansiolíticos por inibição da atividade neural de neurônios límbicos e septais laterais através de canais de GIRK (G-protein-gated inwardly rectifying K+). Por outro lado, a ativação de autoreceptores 5-HT_{1A} pré-sinápticos nos núcleos da rafe

inibem as atividades dos receptores 5-$HT_{2A/2C}$, 5-HT_3 e 5-HT_7, que estão implicadas na geração de depressão e / ou ansiedade (SHIMIZU e OHNO, 2013).

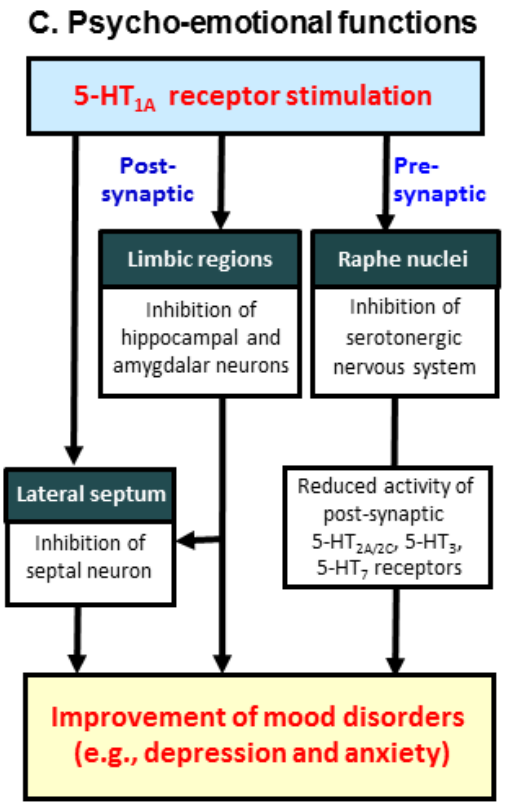

FIGURA 2. Mecanismo de ação de agonistas 5-HT_{1a} na modulação da função psico-emocional. Adaptado de: SHIMIZU e OHNO (2013).

Com a degeneração progressiva dos neurônios dopaminérgicos na DP e formação de DA proveniente de levodopa exógena (L-DOPA) cada vez mais há lugar para terminais nervosos serotonérgicos estriatais. Assim, os agentes farmacológicos que afetam a atividade do impulso nervoso serotonérgico, interagindo com autoreceptores 5-HT_{1A}, podem regular a liberação da serotonina em condições normais e de liberação de DA durante o tratamento com L-DOPA em modelos animais parkinsonianos. Nas últimas circunstâncias, a droga que estimula esses autoreceptores tenderia a atenuar o pico de concentrações estriatais de DA. Se os agonistas 5-HT_{1A} produzem os mesmos efeitos farmacológicos em pacientes com DP, então uma redução no pico dose - discinesias e flutuações *wearing-off* poderia ser esperado (BARA-JIMENEZ *et al*., 2005).

Agonistas de receptores 5-HT_{1A} parecem exercer ações antiparkinsonianas, pelo menos parcialmente através da ativação de receptores pós-sinápticos 5-HT_{1A},

sendo provável que a ação benéfica de agonistas de 5-HT_{1A} é mediada no córtex cerebral e corpo estriado, provavelmente por inibição da atividade neuronal nestas regiões. Além de melhoras motoras, agonistas totais e parciais de receptores 5-HT_{1A} produziram efeitos ansiolíticos em vários modelos animais, que seriam revertidos com antagonistas desses receptores (SHIMIZU e OHNO, 2013).

O ansiolítico não-benzoadiazepínico buspirona também tem sido utilizado para tratar a ansiedade na DP. A buspirona é um agonista parcial de receptores 5-HT_{1A}. A ação ansiolítica da buspirona pode resultar da diminuição da ativação de receptores serotoninérgicos dos tipos 5-HT_{2A} e 5-HT_{2C}, imitando os efeitos da ritanserina, um antagonista pós-sináptico do receptor 5-$HT_{2A/2C}$ (BOND *et al*., 2003). Quando utilizado em doses baixas, buspirona mostra os efeitos ansiolíticos e também reduz discinesias induzidas por L-DOPA, sem causar sedação ou déficit motor (BONIFATI et al., 1994). Embora bem tolerada, a buspirona em doses elevadas pode piorar os sintomas motores da DP, além de causar náuseas e insônia (PREDIGER *et al.,* 2012).

1.3 MODELOS ANIMAIS DE DP

1.3.1 Modelo animal da 6-hidroxidopamina

A neurotoxina 6-hidroxidopamina (6-OHDA) é estruturalmente semelhante à DA e NA e possui uma elevada afinidade para os transportadores de membrana destas catecolaminas (MEREDITH *et al.*, 2008). As primeiras observações dos efeitos biológicos da 6-OHDA mostraram que este agente foi capaz de induzir uma depleção longa e duradoura de NA em nervos simpáticos no coração. Alguns anos mais tarde foram demonstrados que a injeção de 6-OHDA na SNpc era capaz de causar degeneração anterógrada dos neurônios dopaminérgicos nigroestriatal, gerando assim o primeiro modelo animal de DP. Desde esta descoberta, a 6-OHDA tem sido amplamente utilizada para observar o parkinsonismo em animais (LIMA *et al*., 2006).

Pelo fato da 6-OHDA não atravessar a barreira hematoencefálica, deve ser administrada diretamente no encéfalo, por meio de cirurgia estereotáxica (DAUER & PRZEDBORSKI, 2008). Quando injetada na substância negra, acumula-se seletivamente em neurônios dopaminérgicos e os mata devido a toxicidade

envolvida com a geração de radicais livres. As lesões provocadas por essa neurotoxina não resultam em corpúsculos de Lewy na substância negra e pode produzir danos não específicos em outros neurônios (BEAL, 2001).

Quando a 6-OHDA é injetada no estriado, resulta em lesões parciais mais restritas, e seu uso tem oferecido um modelo viável para estudar os sintomas não motores (BRANCHI *et al*. , 2008.; TADAIESKY *et al*., 2008;. SANTIAGO, 2010; DUNNET e LINDGREN, 2012).

A 6-OHDA representa um composto ideal para uso experimental, através do fornecimento de um método econômico e fácil de executar para modelar a doença em roedores, e para investigar novos compostos neuroprotetores para os neurônios dopaminérgicos (PIENAAR, LU e SCHALLERT, 2012).

1.3.2 Modelo animal da rotenona

Um composto extraído de plantas leguminosas que é amplamente utilizado como inseticida e para controlar populações de peixes, a rotenona, inicialmente empregada pela via intranigral, revelou-se um modelo de DP (SANDERS e GREENAMYRE, 2013). No entanto, a injeção intraperitoneal crônica diária de rotenona na forma de um óleo natural tem sido relatado como capaz de induzir deficiências locomotoras e alterações neuroquímicas características de DP. Por ser lipofílica, é capaz de atravessar a barreira hematoencefálica (ALAM e SCHMIDT, 2004).

A rotenona possui como mecanismo de ação a inibição do complexo I da cadeia transportadora de elétrons mitocondrial, o que acarretaria em uma redução na síntese de ATP e a formação de ROS que danificam o próprio complexo I e outras macromoléculas celulares (SANDERS e GREENAMYRE, 2013; BASSANI *et al.*, 2014). Além disso, por meio da produção de ROS a rotenona ativa células da micróglia contribuindo para o dano oxidativo em áreas seletivas do encéfalo (SHERER *et al.*, 2002).

O uso crônico de rotenona em roedores possui como vantagem a geração das inclusões positivas para α-sinucleína e ubiquitina, sendo que os modelos clássicos de DP como a 6-OHDA e MPTP não apresentam tal característica (BOVÉ e PERIER, 2012). As principais limitações do modelo da rotenona podem ser indicadas: a variabilidade na percentagem de animais que desenvolvem uma lesão

nigroestriatal dopaminérgica, a magnitude e localização da lesão e a sua distribuição dentro do corpo estriado (CANNON, *et al*., 2009).

2 OBJETIVOS

2.1 OBJETIVO GERAL

Investigar o envolvimento de neurotransmissores em comportamentos relacionados à ansiedade, por meio da análise comportamental e neuroquímica em modelos animais da DP induzidos por 6-hidroxidopamina (6-OHDA) ou rotenona em ratos.

2.2 OBJETIVOS ESPECÍFICOS

- Avaliar parâmetros relacionados a atividade locomotora após a administração de 6-OHDA ou rotenona através do teste do campo aberto, após 1 e 21 dias de sua administração.
- Avaliar a resposta de congelamento condicionado de animais submetidos ao teste de medo condicionado contextual após 21 dias da lesão por 6-OHDA ou rotenona.
- Avaliar parâmetros relacionados a ansiedade no labirinto em cruz elevado após 21 dias das lesões.
- Quantificar os níveis de serotonina (5-HT), noradrenalina (NA), dopamina (DA) e seus principais metabólicos (HVA e DOPAC) no córtex pré-frontal, amígdala e estriado, através da análise neuroquímica, utilizando a técnica de cromatografia líquida de alta performance (HPLC), após a administração das neurotoxinas e testes comportamentais.
- Correlacionar os resultados obtidos nos testes labirinto em cruz elevado, medo condicionado contextual e campo aberto com os valores encontrados na análise neuroquímica.

3 ARTIGO CIENTÍFICO

Os materiais e métodos, resultados e discussão do trabalho encontram-se no artigo científico a seguir.

Anxiogenic- like behavior induced by 6-OHDA, but not rotenone animal models of Parkinson's Disease may be related to increased serotonin level in amygdala.

Running title: Anxiety- like behavior in animal models of PD.

Jeane Cristina F. Vieira[1], Taysa B. Bassani[1], Ronise M. Santiago, Janaína M. Zanoveli[1], Claudio da Cunha[1], Maria A. B. F. Vital✉[1].

[1]Pharmacology Department, Federal University of Paraná, Brazil

Corresponding author:

✉ Maria Aparecida Barbato Frazão Vital

Departamento de Farmacologia

Universidade Federal do Paraná

Phone: +55 41 3361 1717

Fax: +55 41 3266-2042

Email: vital@ufpr.br

ABSTRACT

Besides motor symptoms, Parkinson's disease (PD) is characterized by non-motor symptoms symptoms which are common and often include depression and/or anxiety disorders. The anxiety in PD may represent a phychological reaction to the development of other symptoms during disease progression, but there is evidence that an increase in anxiety disorders may be related to neurochemical changes in PD. The present study addresses the question whether dopamine, noradrenaline and serotonin levels in brain structures related to PD and anxiety are responsible for anxiety-like behavior in animal models of PD. Two animal models of PD were performed: 6-hydroxydopamine (6-OHDA) intranigral injection bilaterally or rotenone intraperitoneal (i.p.) for 10 days. The elevated plus maze (EPM) and the contextual fear conditioning (CFC) were used to evaluate anxiety-like behaviors (21 days and 24 days after neurotoxins exposure, respectivelly). These tests were performed after motor behavioral impairments were spontaneously reverted on the last motor assessment (day 21). Our data showed that the 6-OHDA model induced anxiogenic-like behavior in the EPM and CFC test. Interestingly, neurochemical analysis indicated an increase in the serotonin (5-HT) levels in the amygdala. For the rotenone model, our results did not reveal alterations on anxiety-like behaviors, neither neurochemical changes in the serotonin levels of amygdala. Together, these results indicate that the model of 6-OHDA in rats presents anxiogenic-like behavior which may be related to the increased serotonin levels in the amygdala.

Keywords: Parkinson’s disease; anxiety; serotonin; 6-OHDA; rotenone.

Abbreviations: CFC, contextual fear conditioning, DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; EDTA, ethylenediaminetetraacetic acid; EPM, elevated plus maze; GAD, generalized anxiety disorder; HVA, homovanillic acid; HPLC, high-performance liquid chromatography; NA, noradrenaline; NET, noradrenalinergic transporter; OFT, open field test; 6-OHDA, 6-hydroxydopamine; PD, Parkinson’s disease; 5-HT, 5-hydroxytryptamine (serotonin); SNpc, substantia nigra pars compacta.

1. Introduction

The existence of non-motor symptoms in Parkinson's Disease (PD) was found to be an important factor in reducing the patient's quality of life. Depression and anxiety were included in this category of symptoms and its coexistence often occurs (Negre-Pages et al., 2010; Pachana et al., 2013). Despite the development of research dedicated to the non-motor symptomatology of PD, literature focused on the anxiety disorders among individuals or animal models of PD remains sparse (Dissanayaka et al., 2014; Prediger et al., 2012). Recent studies indicate that the prevalence of anxiety disorders in PD varies from 12.8% to 43.0% (Dissanayaka et al., 2010; Leentjens et al., 2011; Marinus et al, 2002; Ozdilek et al., 2012) occuring more frequently in parkinsonian population than in matched controls or other medically ill patients (Bogdanova and Cronin-Golomb, 2012; Picillo et al., 2013). The most common disorders found are generalized anxiety disorder (GAD), social phobia, panic disorder and non-specific anxiety (Bolluk et al., 2010; Dissanayaka et al., 2010; Lauterbach et al., 2003; Leentjens et al., 2011).The impact of anxiety in individuals with PD has not been well established due to difficulties in recognize this in clinical practice because of factors as different methods used by clinicians and patients' reluctance to report psychological symptoms (Dissanayaka et al., 2014; Kano et al., 2011; Leentjens et al., 2011).

Initially, it was suggested that anxiety disorders might be related only to the psychological reaction to the development of other symptoms during the progress of the disease. However there are evidence that may be an association to neurochemical changes in PD (Prediger et al., 2012) even though some authors proposed that treatment with antiparkinsonian medications could induce or complicate this anxiety (Richard et al., 1996; Vázquez et al., 1993; Walsh and Bennett, 2001). Motor symptom fluctuations from "wearing off" of pharmacological treatment were related to anxiety disorders (Kulisevsky et al., 2007; Pontone et al, 2009) even though the presence of anxiety in PD could have some biological mechanisms that lead to its occurrence at any stage of PD (Kano et al., 2011; Martinez-Martin and Damian, 2010). It is important to highlight that in the Braak stages of PD (Braak et al., 2004) was observed neurodegeneration of serotoninergic neurons present in raphe nuclei earlier than nigroestriatal dopaminergic loss. In this process, structures including the anterior olfactory structures, amygdala, dorsal motor

nucleus of vagus, locus coeruleus, hippocampus and cerebral cortex are compromised. Also the progressive death of midbrain dopaminergic neurons in PD is paralleled by the concomitant degeneration of noradrenergic and serotonergic systems (Braak et al., 2004; Frisina et al., 2009; Halliday, 2012; Kish et al., 2008; Remy et al., 2005).

Previous findings on anxiety-like behaviors in animal models of PD have been limited and not consensual regardless of anxiety being a common non-motor symptom in PD, as well as depression. With regard to 6-hydroxydopamine (6-OHDA) neurotoxin, some studies are controversial probably by using different methodologies. Branchi et al., (2008) claim that anxiety does not seem to be a common feature of this PD model and showed that the 6-OHDA injury produced a 36% loss of striatal dopamine levels and a reduction in anxiety-like behavior in rats. Nevertheless there are studies that reported an anxiogenic-like behavior inflicted by 6-OHDA model (Bonito-Oliva et al., 2014; Campos et al., 2013; Carnicella et al.; 2014;Chen et al., 2011; Tadaiesky et al., 2008) whose lesions were performed in the striatum or the Substantia nigra *pars compacta* (SNpc) but with differents protocols.

Considering the controversies and lack of consense in this field, the aim of the present study was to investigate the involvement of monoamines in brain areas related to the PD and anxiety-like behaviors such as striatum, prefrontal cortex and amygdala. Moreover, we investigated the anxiety-like behaviors in two animal models of parkinsonism. One model was based on intranigral bilateral injection of the neurotoxin 6-OHDA. The other model was based on short–term intraperitoneal (i.p.) administration of the pesticide rotenone.

2. Material and Methods

2.1 Animals

Male Wistar rats from our breeding colony weighing 280–320 g at the beginning of the experiments were used. The animals were randomly housed in groups of five in polypropylene cages with wood shavings as bedding and maintained in a temperature-controlled room (22±2°C) on a 12-h light-dark cycle (lights on at 7:00 a.m.). The animals had free access to water and food throughout the experiment. The studies were carried out in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animals, United States National Institute of Health. In addition, the protocol complies with the recommendations of

Federal University of Paraná and was approved by the University Ethics Committee (Protocol Nº 697).

2.2 Drugs

Rotenone and 6-OHDA were purchased from Sigma Chemical, St. Louis, MO, USA. The neurotoxin 6-OHDA was prepared according to established concentration: 6 µg in 1 µL of artificial cerebrospinal fluid (aCSF), supplemented with 0.2% ascorbic acid; Sigma, St. Louis, MO, USA (Ferro et al., 2005; Lima et al., 2006). Rotenone was dissolved in sunflower oil at a final concentration of 2.5 mg/mL and administered i.p. at a dose of 2.5 mg/Kg and the control group received the rotenone vehicle at a dose of 1 ml/kg, i.p. as described by Morais et al. (2012) and Bassani et al. (2014).

2.3 Stereotaxic surgery

The animals were anesthetized with equitesin (chlornembutal, 0.3 ml/kg, intraperitoneal - i.p.) and atropine sulphate (0.4 mg/kg, intraperitoneal [i.p]). Bilateral infusions of 6-OHDA were performed through a 27-gauge stainless steel needle, according to the following coordinates: anteroposterior (AP): −5.0 mm from the bregma; mediolateral (ML): ±2.1 mm from the midline; dorsoventral (DV): -8.0 mm from the skull (Paxinos and Watson, 2005). The control of the flow of the injections were made by using an electronic pump (Harvard Apparatus, USA) at a rate of 0.33 µL/min, for 3 min, followed by 2 min with the needle in the injection site to avoid reflux. Sham operations followed the same procedure but aCSF was injected instead. After the surgery these rats also received penicillin G-procaine (20.000 U in 0.1 ml, intramuscular) to avoid infections.

2.4 Experimental design

Experiment 1: Rats were distributed randomly into 2 groups: sham and 6-OHDA. After stereotaxic surgeries that allowed the intranigral injections of 6-OHDA open-field test was conducted in the subsequent 1 and 21 days. In addition, the same animals were tested in the elevated plus maze (EPM) 21 days after the neurotoxins exposures. These groups were analysed in contextual fear conditioning (CFC) in the day 24 (Conditioning session performed in day 23).

Experiment 2: Rats were distributed randomly into 2 groups: vehicle and rotenone. For 10 consecutives days, rotenone dissolved in sunflower oil (2.5 mg/Kg)

or only its vehicle (sunflower oil, 1 mg/Kg) were administered intraperitoneally. After the last rotenone exposure, the open field test was performed 1 and 21 days. Besides, in 21 day after exposures these animals were analysed in the EPM. These groups were analysed in CFC in the day 24 (Conditioning session performed in day 23).

At the end of experiments 1 and 2, the rats were decapitated followed by dissection of the striatum, prefrontal cortex and amygdala structures for neurochemical purposes.

2.5 Behavioral testing

The behavioral assessment of anxiety-like parameters was performed 21 and 24 days after the last neurotoxin injections to allow a complete recovery from hypolocomotion what was observed in previous studies from our group (Bassani et al., 2014; Santiago et al., 2010). Tests behaviors were recorded with a digital video camera.

2.5.1 Open-field test

The apparatus consists of a round wood box with a diameter of 97 cm and circular wall of 42 cm high. The arena floor was divided into three concentric circles. The number of areas in the inner circle, middle and outer circles was 1, 6 and 12, respectively. The animals were gently placed in the in the center of the open-field and were allowed to freely explore the area for 5 min.Hand-operated counters were used to score two motor parameters: locomotion frequency (number of crossings from one section to the other) and rearing frequency (number of times the animals stood on their hind paws). The open-field was washed with a 5% water-ethanol solution before behavioral testing to eliminate possible bias due to odors left by previous rats.

2.5.2 Elevated plus maze test

The elevated plus maze consists of two open arms (50 cm × 10 cm) that were arranged to form a plus shape with two closed arms of same dimension and 50 cm high walls. The entire apparatus was elevated 50 cm above the ground. To avoid the fall of rat, a rim of Plexiglas (0.5 cm high) surrounded the perimeter of the open arms. The protocol used was previously described in the literature (Pellow et al., 1985; Walf

and Frye, 2007), and the animal's exposure to EPM occurred only on the test day. Animals were placed in the central junction facing an open arm, and allowed to explore for 5 minutes. Before the next animal tested, the maze was wiped clean with a 5% ethanol solution. Standard measures for testing were recorded in the EPM: percentage (%) time in open arms [time spent in arms (s)/300 (s)) x100], % open arms entries, closed arms entries and total arms entries.

2.5.3 Contextual fear conditioning test

Briefly, the conditioning chamber consists of a box (26 x 3.5 x 21cm; Insight, Ribeirão Preto, SP, Brazil). Three sides of the enclosure are made of steel and the fourth side is acrylic, which allows the animal's behavioral analysis. The bottom of the box is fine spaces of metal connected to an electrical stimulator that can release electric discharges in the animal paws. The contextual fear conditioning (training session) was conducted as described in the literature (Ninomiya et al., 2010). Conditioning session: Rats were placed for 3 minutes in the conditioning chamber and then received an electric foot shock (1.5 mA, lasting 1s). Later, the animals remained in the box for 1 minute, then returned to their respective nursery boxes. In the next day, the test session was performed.

Test session: The animals were re-exposed to environmental chambers for 9 minutes without receiving the electric foot shock (Session test). The time of freezing (in seconds) of each animal behavior assessed in the test session was used as an anxiety scores (contextual fear conditioning). One animal was found to freeze when it presented a stereotyped squat position with complete immobility, except for breathing movements.

2.6 Determination of dopamine and metabolites, noradrenaline and serotonin concentrations

The striatum, amygdala and prefrontal cortex structures were dissected and stored at -80ºC until the neurochemical quantification. The endogenous concentrations of DA, DOPAC, HVA, NA and 5-HT were assayed by reverse-phase high performance liquid chomatography (HPLC) with electrochemical detection (ED). Briefly, the system consisted of a Synergi Fusion-RP C-18 reverse-phase column (150 x 4.6 mm i.d., 4 m particle size) fitted with a 4 x 3.0 mm pre-column (Security Guard Cartridges Fusion-RP); an electrochemical detector (ESA Coulochem

Electrochemical Detector) equipped with a guard cell (ESA 5020) with the electrode set at 350 mV and a dual electrode analytical cell (ESA 5011A); a LC-20AT pump (Shimadzu) equipped with a manual Rheodyne 7725 injector with a 20 µL loop. The column was maintained inside in a temperature-controlled oven (25ºC; Shimadzu). The cell contained two chambers in series: each chamber including a porous graphite coulometric electrode, a double counter electrode and a double reference electrode. Oxidizing potentials were set at 100 mV for the first electrode and at 450 mV for the second electrode. The tissue samples were homogenized with an ultrasonic cell disrupter (Sonics) in 0.1 M perchloric acid containing sodium metabisulfite 0.02% and internal standard. After centrifugation at 10,000x*g* for 30 min at 4ºC, 20 µL of the supernatant was injected into the chromatograph. The mobile phase, used at a flow rate of 1 mL/min, had the following composition: 20 g citric acid monohydrated (Merck), 200 mg octane-1-sulfonic acid sodium salt (Merck), 40 mg ethylenediaminetetraacetic acid (EDTA) (Sigma), 900 mL HPLC-grade water. The pH of the buffer running solution was adjusted to 4.0 then filtered through a 0.45 m filter. Methanol (Merck) was added to give a final composition of 10% methanol (v/v). The neurotransmitters and metabolites concentrations were calculated using standard curves that were generated by determining in triplicate the ratios between three different known amounts of the internal standard. The unit was expressed as ng/g of wet weight.

2.7 Statistical analysis

All data were analyzed using the program GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, USA) and STATISTICA 7.0 (StatSoft Iberica Inc.). Levene test and Kolmogorov was initially employed to ensure that the data obtained after the studies conducted in groups meet the necessary criteria of normality for applying parametric tests. Data presented normal distribution and expressed as mean ± standard error of the mean (SEM). Analysis of locomotor activity data were subjected to two-way analysis of variance (ANOVA) followed by Newman-Keuls post hoc test, when appropriated. The number of circles crossed or rearing was dependent factors and the condition (control or lesioned groups) and time as independent factors. Furthermore, in both experiments the unpaired Student's *t*-test was used to analyse the EPM parameters % time spent in the open arms, total number of entries in the arms, % open arms entries and number of entries in the

closed arms. For the contextual fear conditioning test the freezing response (in seconds) was considered the dependent factor and the condition (control or lesioned groups) as the independent factor, Student's *t*-test was also applied. Neurochemical analysis were performed by Student's *t*-test. A P value ≤ 0.05 was considered significant.

3. Results

3.1 Open-field test

Locomotion activities obtained after exposures to neurotoxins in 1 day, as can be seen in Figure 1A and C, show a significant reduction in the group 6-OHDA or rotenone in comparison to sham vehicle groups, respectivally. Significant decrease of locomotion in the 6-OHDA group was observed in 1 day after the surgery compared with sham ($P < 0.01$; Fig. 1A), was indicated by the condition factor [$F(1.0) = 9.99$; $P = 0.034$], interaction [$F(1,0) = 5.49$; $P = 0.02$] and by the time [$F(1,0) = 6.62$; $P = 0.01$] factors. In the rotenone group this reduction on locomotor activity was demonstrated by the condition factor [$F(1.0) = 14.39$; $P = 0.0006$], time [$F(1.0)= 7.71$; $P =0.01$] but not by the interaction [$F(1.0) = 3.438$; $P = 0.07$] factors. Nevertheless, after 21 days of neurotoxins injections no difference between these animals and sham or vehicle ones was observed. Rearing frequency was decreased after 1day of lesions and a recovery of this feature after 21 days to both models (Figure 1B and D). In the 6-OHDA group, this impairment was demonstrated by the condition factor [$F(1.0) = 12.85$; $P = 0.01$], interaction [$F(1.0) = 11.67$; $P = 0.02$] but not by the time [$F(1.0) = 0.33$; $P = 0.57$] factors. With regard of rotenone, this reduced was demonstrated by thecondition factor [$F(1.0) = 5.82$; $P = 0.02$], time [$F(1.0) =26.09$; $P <0.0001$], but not by the interaction [$F(1.0) = 3.99$; $P = 0.05$] factors. Post hoc analysis demonstrated no difference between condition in 21 days ($P>0.05$) to both models and all parameters observed in OF test.

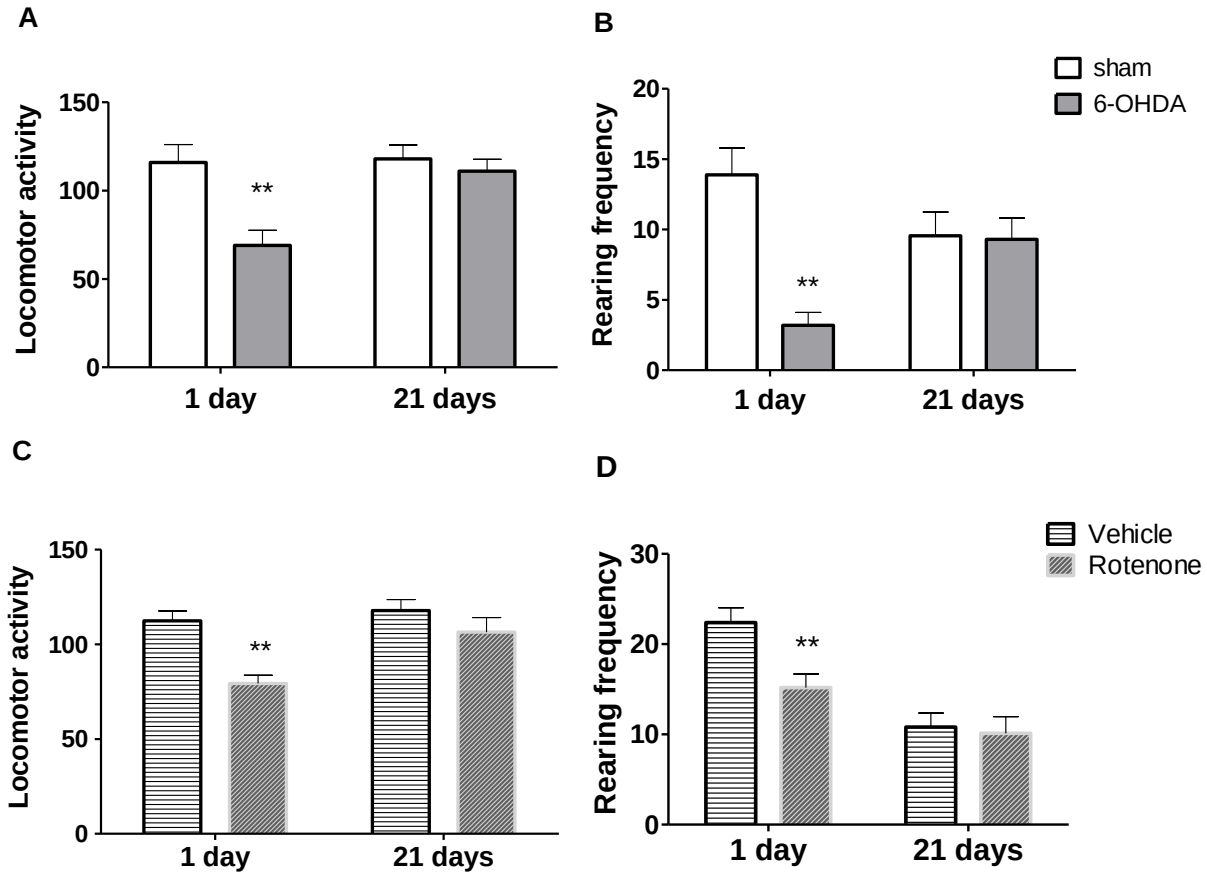

Figure 1. Motor behavior alterations after 1 day and 21 days 6-OHDA or rotenone administrations. (A) Locomotion activity of 6-OHDA model. (B) Rearing frequency of 6-OHDA model. (C) Locomotion activity of rotenone model. (D) Rearing frequency of rotenone model. The values are expressed as mean ± SEM (n=9-10/ group). Differences between groups indicated by **: different from the sham or vehicle (P<0.01) groups 1 day after exposure; Two-way ANOVA followed by Newman-Keuls test.

3.2 Elevated plus maze test

In the 6-OHDA model was observed a decreased of time in the open arms compared with sham group (Figure 2A) (t =3,22(14), P = 0.006). As for the other parameters, there was no statistically significant difference between the groups to be described an effect (Figure 2B, C, D).

On the other hand EPM did not indicate a difference in the rotenone groups (Figure 3) to all measures. The test session was performed in day 21 after the last day of neurotoxin exposures so as demonstrated by OF test and a measure of spontaneous motor activity in EPM (total and/or closed arm entries) this parameter did not interfere in this test.

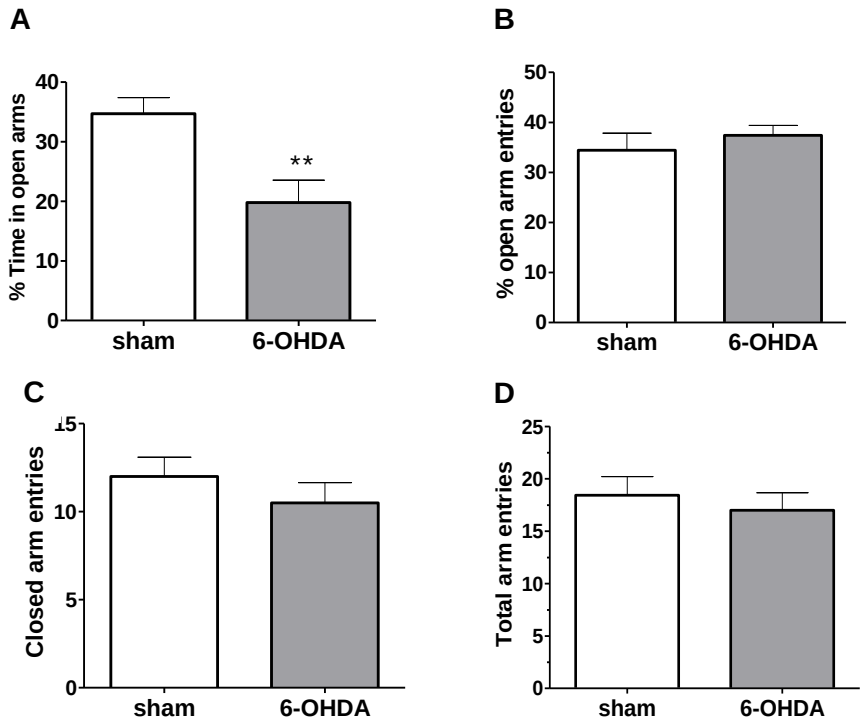

Figure 2. Effect of lesion with 6-OHDA in animals submitted to the EPM test assessed by parameters of space-time activities, 21 days after lesion. (A) % Time spent in open arms. (B) % of entries into the open arms. (C) Number of entries into the closed arms. (D) Total number of entries in all arms. Data represented by ± SEM (n=8-10/ group).** P <0.01 (compared with sham group, Student's *t*-test).

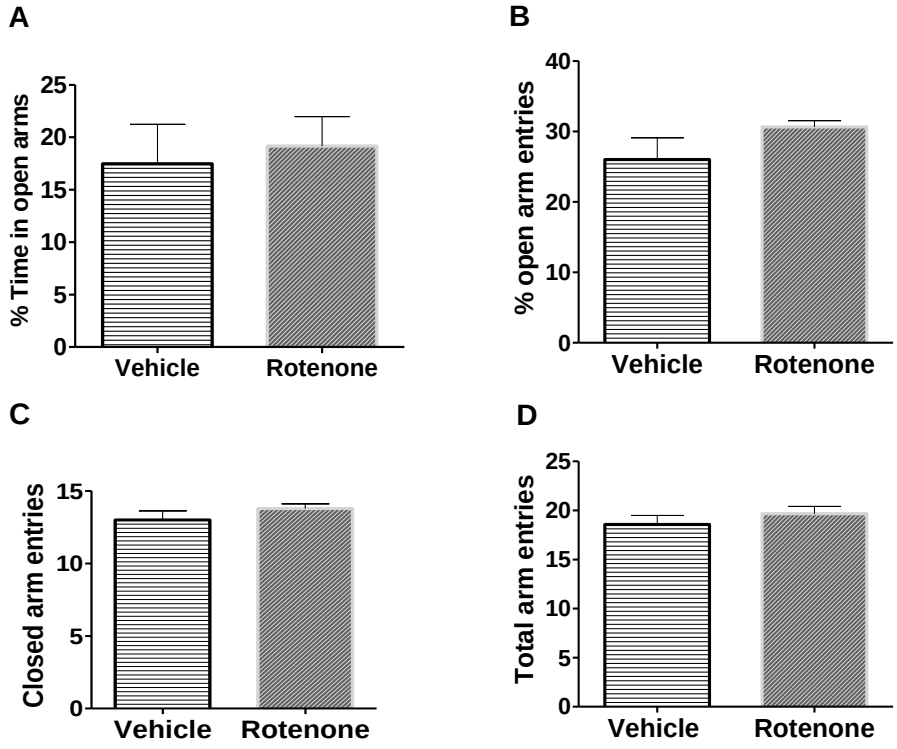

Figure 3. Effects of lesion with rotenone in animals submitted to the EPM test assessed by parameters of space-time activities, 21 days after lesion. (A) % Time spent in open arms. (B)

% of entries into the open arms. (C) Number of entries into the closed arms. (D) Total number of entries in all arms. Data represented by ± SEM (n=7-9/ group, Student's *t*- test).

3.3 Contextual fear conditioning test

The parameter freezing time (seconds) as illustrated in Figure 4A, was significantly increased in rats from the 6-OHDA group than sham animals (t = 2.98 (13), P =0.01). This effect was observed for rotenone model that demonstrated no difference between vehicle and rotenone groups (t = 0.88 (14), P =0.39) (Figure 4B).

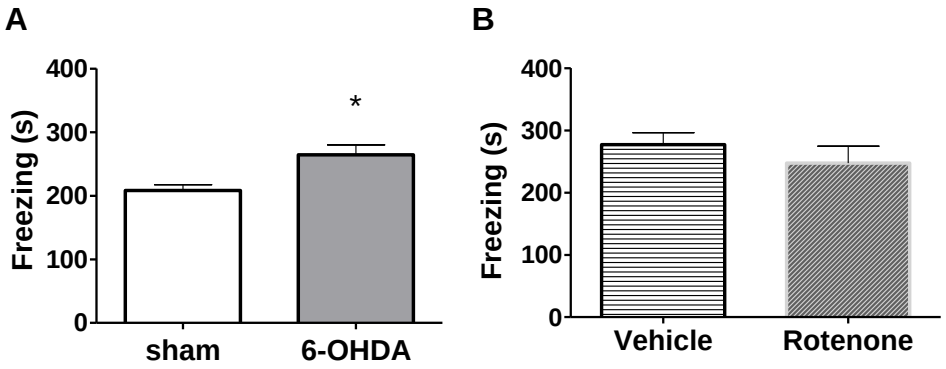

Figure 4. Freezing behavior time (seconds) 21 days after lesions. (A) Anxiogenic-like effect in 6-OHDA animals. * P <0.05 (compared to the sham group, Student's *t*-test). (B) No effect of freezing in rotenone animals compared to control (vehicle). Data represented by ± SEM (n= 8/group).

3.4 Determination of dopamine and metabolites, noradrenaline and serotonin concentrations

Dopamine levels was found significantly reduced in striatum, prefrontal cortex and amygdala to both models (P<0.01). Moreover, dopamine metabolites, DOPAC and HVA were decreased in this same structures and groups (P<0.01), only with a difference of P<0.05 in amygdala from 6-OHDA group (Table 1) and striatum and prefrontal cortex from the rotenone group which concern to DOPAC (Table 2). Noradrenaline decreased significantly (P<0.01) in both models (Table 3 and 4). In serotonin analysis was observed an increase of its content in the amygdala from 6-OHDA group compared to sham (t = 3.10 (12), P =0.09) (Table 3) what not occured in rotenone groups (t = 0.06 (14), P =0.95) (Table 4). Reductions of serotonin in prefrontal cortex and striatum were verified in both neurotoxins lesioned animals (P<0.01).

Table 1. Neurochemical quantification of striatum, prefrontal cortex and amygdala concentrations of dopamine and metabolites DOPAC and HVA (expressed as ng/g tissue) in the 6-OHDA model. The values are expressed as mean ± SEM (n=8-11/ group). Differences between groups are indicated by: ** different from sham (P<0.01); * different from sham (P<0.05). Student's *t*-test.

	Dopamine		DOPAC		HVA	
Areas	**sham**	**6-OHDA**	**sham**	**6-OHDA**	**sham**	**6-OHDA**
Striatum	4146.0±335.5	1340.0±234.1**	2804.0±191.1	1929.0±230.1**	374.6±23.5	212.1±13.8**
Prefrontal cortex	16.7±1.6	7.7±0.5**	9.4±0.8	5.5±0.4**	17.2± 2.7	9.1±0.8**
Amygdala	110.2±14.3	56.8±11.3**	134.6±18.3	82.3±11.6*	19.1± 1.3	8.4± 1.5**

Table 2. Neurochemical quantification of striatum, prefrontal cortex and amygdala concentrations of dopamine and metabolites DOPAC and HVA (expressed as ng/g tissue) in the rotenone model. The values are expressed as mean ± SEM (n=8-11/ group). Differences between groups are indicated by: ** different from vehicle (P<0.01); * different from vehicle (P<0.05). Student's *t*-test.

	Dopamine		DOPAC		HVA	
Areas	**Vehicle**	**Rotenone**	**Vehicle**	**Rotenone**	**Vehicle**	**Rotenone**
Striatum	3595.0±147.2	2176.0± 176.1**	8936.0 ± 2236.0	1537.0 ±960.0*	64.3± 4.3	37.4± 3.0**
Prefrontal cortex	18.4± 1.9	12.4± 1.0**	46.0±5.4	28.6± 4.7*	17.5± 1.4	10.3± 1.3**
Amygdala	212.8± 12.0	93.6± 14.9**	468.7± 50.0	101.4± 17.7**	32.1± 4.9	15.2± 3.0**

Table 3. Neurochemical quantification of striatum, prefrontal cortex and amygdala concentrations of noradrenaline and serotonin in the 6-OHDA model (expressed as ng/g tissue). The values are expressed as mean ± SEM (n=8-10/ group). Differences between groups are indicated by: ** different from sham (P<0.01); Student's *t*-test.

	Noradrenaline		5-HT	
Areas	**Sham**	**6-OHDA**	**sham**	**6-OHDA**
Striatum	85.9 ± 5.1	11.8 ± 2.3**	133.6 ± 13.8	70.1 ± 2.6**
Prefrontal cortex	178.2 ± 10.2	83.1 ± 8.2**	178.4 ± 11.2	89.5 ± 9.2**
Amygdala	116.0 ± 14.0	34.5± 4.8**	40.5 ± 6.3	70.5±7.3**

Table 4. Neurochemical quantification of striatum, prefrontal cortex and amygdala concentrations of noradrenaline and serotonin in the rotenone model (expressed as ng/g tissue). The values are expressed as mean ± SEM (n=8-10/ group). Differences between groups are indicated by: ** different from vehicle (P<0.01); Student's *t*-test.

	Noradrenaline		5-HT	
Areas	**Vehicle**	**Rotenone**	**Vehicle**	**Rotenone**
Striatum	264.0± 54.7	77.6± 11.8**	11.4 ± 1.6	4.1 ± 1.3**
Prefrontal cortex	371.9± 30.9	233.5± 16.3**	9.1± 1.6	1.8 ± 0.5**
Amygdala	308.7± 26.8	190.2± 26.3**	2.7 ± 0.4	2.8 ± 0.7

4. Discussion

Our data suggested an anxiogenic-like behavior in 6-OHDA lesioned rats observed in EPM and CFC tests after 21 days of toxin injection and this effect may be related to an increased of serotonin in amygdala. In OF test, hypolocomotion was spontaneously reversed after 21 days in both lesioned animals possibly because of plasticity events that balance neuronal death. In EPM test, measure of spontaneous locomotor activity (total and/or closed arm entries) confirmed what was observed in the OF test (day 21).

These data are in agreement with others from literature. Our experimental protocol consisted on direct bilateral injections of 6-OHDA into the SNpc, which yields a faster lesion of dopaminergic cell bodies, followed by degeneration of striatal terminals. Corroborating with our protocol, Carnicella et al. (2014) observed a decrease of time spent in open arms of animals lesioned bilaterally in SNpc (6 µg) after 3 weeks. Even though Tadaiesky et al., (2008) used a 6-OHDA concentration of 12 µg per injection site bilaterally into the ventrolateral area of the dorsal striatum they also observed an anxiogenic-like behavior in EPM, 3 weeks after surgery. Supporting partially this results, Chen et al.,(2011) performed the injection of 6-OHDA (10.5 µg) into bilateral dorsal striatum and obtained a 31.5% loss of dopaminergic neurons in the SNpc accompanied by anxiogenic-like behavior 15 days after surgery. Although Bonito-Oliva et al. (2014) used a mouse model of 6-OHDA in the dorsal striatum they also observed an anxiogenic-like effect, 3 weeks after surgery in EPM. While striatal injection of 6-OHDA usually produces partial DA cell loss corresponding to the early stage of the disease (Breit et al., 2007), the non-motor symptoms are not evident in some studies (Branchi et al., 2008) but evident in others (Carnicella et al., 2014; Chen et al., 2011; Tadaiesky et al., 2008). In contrast, the bilateral injection of 6-OHDA (10.5 µg) into the dorsal striatum of rats performed by Branchi et al. (2008), resulted in a reduction in anxiogenic-like behavior tested in the EPM 5 weeks after surgery. This controversy effects could be attributed to differences in the local of injection, employed doses besides the age of the animals and timeline of observations. Also the chosen lesion proportion seems to influence the features observed in animals. The 6-OHDA lesion of the medial forebrain bundle (MFB) is a protocol used in the study of anxiety in animal models of PD, however presented discrepancy data. Some authors related no anxiety-like behaviors

(Carvalho et al., 2013; Delaville et al., 2012) and others reported anxiogenic-like behaviors in anxiety tests including the EPM test (Eskow Jaunarajs et al., 2010; Hui et al., 2014; Jungnickel et al., 2011; Sun et al., 2015) that may be related to monoamines alterations.

The present study revealed a significant decrease of DA and metabolites in striatum, prefrontal cortex and amygdala in both PD animal models tested (Table 1 and 2). Previous studies of our group showed that the protocol used for 6-OHDA lesions was able to reduce about 28.1% of the neurons located at the SNpc when compared to sham group ($P<0.01$) and a consequently average 44% reduction of striatal dopamine levels (Santiago et al., 2014). The loss of nigrostriatal dopaminergic neurons has been proposed to mediate mood-related disorders in animal models of PD (Drui et al., 2014). Degeneration of the mesolimbic pathway resulting in a dopamine depletion suggest that a dysfunction of the limbic loop linking the basal ganglia to the orbitofrontal cortex could help to promote anxiety in PD (Brichta et al., 2013; Cummings, 1993).

Even though DA many times has been the major neurotransmitter investigated in PD, others have been linked to PD symptoms such as anxiety. The concomitant loss of noradrenergic (NAergic) with dopaminergic (DAergic) neurons in the limbic system, have been indicated as certain contribution for the PD behavioral symptoms. Also striatal DAergic degeneration and NAergic alterations in prefrontal cortex could provoke emotional reactivity and anxiety (Tadaiesky et al., 2008) as we observed in our study that revealed significantly decrease in striatal DA and prefrontal cortex NA levels in both models. According to Espejo (1997), 6-OHDA injected directly in the prefrontal cortex induced a significant anxiogenic-like effect evaluated in the elevated plus maze. Besides, our findings demonstrated a NA loss in striatum showing the impairment of NA system in PD since our 6-OHDA lesion protocol did not utilize desipramine, a noradrenergic transporter (NET) blocker, to protect NAergic neurons. Even though this area is poorly innervated by NAergic fibers, the locus coeruleus (LC) is an important source not only of extracellular NA but also of extracellular DA in the brain, and the inhibition of the activity of the LC by a degeneration of LC NAergic neurons before that of SNpc DAergic neurons (Braak et al., 2004) reduces DA and NA release in the cortex (Devoto et al., 2005; Delaville et al., 2011).

Although two studies observed an anxiogenic-like effect it was demonstrated controversial 5-HT levels in amygdala. Eskow Jaunarajs et al., (2010) described that

amygdalar 5-HT and 5-HIAA was increased in lesioned rats, likely signifying an increase in 5-HT turnover. However, Sun et al.,(2015) demonstrated that 5-HT levels in these brain region were unaffected by 6-OHDA lesion of the MFB. Serotonin is a classical neurotransmitter involved with anxiety that may explain even some pre-motor symptoms of PD due to evidences that serotonergic neuronal loss precedes the dopaminergic loss (Braak et al., 2004; Remy et al., 2005). Early in PD, there is a degeneration in the raphe nucleus, which in turn reduces the 5-HT levels in striatum, with the caudate nucleus apparently more denervated than putamen (Kish et al., 2008; Wilson et al., 1996). This evidence is observed by our results which indicated decrease of 5-HT in striatum of 6-OHDA and rotenone groups (Table 3 and 4).

Otherwise, our data showed an increased in the 5-HT levels into the amygdala complex in 6-OHDA group and no alteration in rotenone group. This brain structure, in particular the basolateral nucleus of the amygdala, has long been involved in the regulation of defensive behavior, and hence in fear and anxiety (Fanselow and Gale, 2003). The affective component of anxiety would be integrated in the amygdala which could be explained by an activation of the dorsal raphe nucleus resulting in increase of 5-HT levels which in amygdala facilitates the defense strategies that are mainly integrated at the amygdala. Simultaneously, the defense reactions organized in the dorsal periaqueductal gray (dPAG) are inhibited characterizing the dual 5-HT-defense hypothesis proposed by Deakin and Graeff (1991) (Graeff, 2002). Therefore, it was suggested that non-motor symptoms are a consequence of DA dysfunction concomitant with NA and/or 5-HT alterations (Delaville et al., 2012).

Concernig to rotenone, previous findings in our group by the same protocol (Morais et al., 2012) or by intranigral rotenone (Santiago et al., 2010) indicated this model adequated to understand depressive-like behaviors, a non-motor feature of PD that often coexists with anxiety. According to these results that demonstrated a non-motor symptom analysis, would be expected that this treatment would be capable to produce anxiogenic-like behaviors, what were not observed in EPM and CMC test. The main limitations of rotenone model such as variability in the percentage of animals that develop a dopaminergic nigrostriatal injury and the magnitude and location of the lesion and its distribution within the striatum can impair its use in non-motor symptoms studies (Cannon et al., 2009).

As mentioned before, 5-HT was not altered in amygdala, a crucial structure to anxiety-like behaviors, in the rotenone group. This interesting finding suggests that

the increased of serotonin in amygdala may play an important role for the development of anxiogenic-like behaviors in animal models of PD.

5. Conclusion

The present data revealed a possible relation between anxiogenic-like behaviors and increased serotonin levels of amygdala complex when 6-OHDA animal model of PD was used. Anxiety-like behaviors and their association with animal models of PD need to be more explored due to the impact of this symptom in the quality of life of the patient. This feature is not entirely understood, but it seems to be related to the serotonin content in limbic areas such as amygdala complex and the relation between other monoamines such as dopamine and noradrenaline.

Conflict of interest

The authors have no conflict of interest to declare.

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References

Bassani TB, Gradowski RW, Zaminelli T, Barbiero JK, Santiago RM, Bochen SL, Cunha Cda, Lima MMS, Andreatini R, Vital MABF. Neuroprotective and antidepressant-like effects of melatonin in a rotenone-induced Parkinson's disease model in rats. Brain research 2014;1595: 95-105.

Bolluk B, Ozel-Kizil ET, Akbostanci MC, Atbasoglu EC. Social anxiety in patients with Parkinson's disease. J Neuropsychiatry Clin Neurosci 2010.; 22:390-394.

Bonito-Oliva A, Masini D, Fisone G. A mouse model of non-motor symptoms in Parkinson's disease: focus on pharmacological interventions targeting affective dysfunctions. Front. Behav. Neurosci. 2014;8:290.

Bogdanova Y, Cronin-Golomb A. Neurocognitive correlates of apathy and anxiety in Parkinson's disease. Parkinsons Disease 2012: 1-9.

Braak H, Ghebremedhin E, Rüb U, Bratzke H, Tredici KD. Stages in the development of Parkinson's disease-related pathology. Cell and tissue research 2004; 318:121-134.

Branchi I, D'Andrea I, Armida M, Cassano T, Pezzola A, Potenza RL, et al. Nonmotor Symptoms in Parkinson's Disease: Investigating Early-Phase Onset of Behavioral Dysfunction in the 6-OHDA Rat Model. Journal of Neuroscience Research2008; 86:2050-2061.

Breit S, Bouali-Benazzouz, R., Popa, R. C., Gasser, T., Benabid, A. L., and Benazzouz, A. Effects of 6-hydroxydopamine-induced severe or partial lesion of the nigrostriatal pathway on the neuronal activity of pallido-subthalamic network in the rat. Exp. Neurol. 2007;205: 36–47.

Brichta L, Greengard P, Flajolet M. Advances in the pharmacological treatment of Parkinson's disease: targeting neurotransmitter systems. Trends in Neurosciences 2013; 36 (9): 543-554.

Cannon JR, Tapias V, Na HM, Honick AS, Drolet RE, Greenamyre JT. A highly reproducible rotenone model of Parkinson's disease. Neurobiology of Disease 2009; 34: 279–290.

Campos FL, Carvalho MM, Cristovão AC, Je G, Baltazar G, Salgado AJ et al. Rodent models of Parkinson's disease: beyond the motor symptomatology. Frontiers in behavioral neuroscience 2013; 7: 1-11.

Carnicella S, Drui G, Boulet S, Carcenac C, Favier M, Duran T and Savasta M. Implication of dopamine D3 receptor activation in thereversion of Parkinson's disease-related motivational deficits. Transl Psychiatry 2014;1-8.

Carvalho MM, Campos FL, Coimbra B, Pego JM, Rodrigues C, Lima R et al. Behavioral characterization of the 6-hydroxidopamine model of Parkinson's disease and pharmacological rescuing of non-motor deficits. Mol. Neurodegener. 2013;8 (14):1-11.

Chen L, Liu J, Ali U, Gui ZH, Houg C, Fan L et al. Chronic, systemic treatment with a metabotropic glutamate receptor 5 antagonist produces anxiolytic-like effects and reverses abnormal firing activity of projection neurons in the basolateral nucleus of amygdala in rats with bilateral 6-OHDA lesions. Brain Res.Bull. 2011;84: 215–223.

Cummings J. Frontal-subcortical circuits and human behavior.Arch Neurol 1993;50:873–880.

Delaville C, De Deurwaerdère P, Benazzouz A. Noradrenaline and Parkinson's Disease. Frontiers in system neuroscience 2011;5:1-12.

Delaville C, Chetrit J, Abdallah K, Morin S, Cardoit L, De Deurwaerdere P, Benazzouz A. Emerging dysfunctions consequent to combined monoaminergic depletions in Parkinsonism. Neurobiol. Dis. 2012; 45: 763-773.

Devoto P, Flore G, Saba P, Fá M, Gessa G. Corelease of noradrenaline and dopamine in the cerebral cortex elicited by single train and repeated train stimulation of the locus coeruleus. BMC Neurosci 2005;6:31.

Dissanayaka NNNW, White E, O'Sullivan JD, Marsh R, Pachana NA, Byrne GJ The Clinical Spectrum of Anxiety in Parkinson's Disease. Mov Disorders 2014; 29: 967-975.

Dissanayaka NNNW, Sellbach A, Matheson S, O'Sulivan JD, Silburn PA, Byrne GJ et al. Anxiety disorders in Parkinson's disease: prevalence and risk factors. Mov Disorders 2010;25:838-845.

Drui G.,Carnicella S,Carcenac C,Favier M,Bertrand A, Boulet S,et al. Loss of dopaminergic nigrostriatal neurons accounts for the motivational and affective deficits in Parkinson's disease. Mol.Psychiatry 2014;19, 358–367.

Eskow Jaunarajs KL, Dupre KB, Ostock CY, Button T, Deak T, Bishop C. Behavioral and neurochemical effects of chronic L-DOPA treatment on nonmotor sequelae in the hemiparkinsonian rat. Behav. Pharmacol. 2010; 21, 627-637.

Espejo EF.Selective dopamine depletion within the medial prefrontal cortex induces anxiogenic-like effects in rats placed on the elevated plus maze. Brain Res 1997;762:281–284.

Fanselow MS, Gale GD.The amygdala, fear, and memory. Ann. N. Y. Acad.Sci. 2003; 985:125–134.

Ferro MM, Belissimo MI, Anselmo-Franci JA, Angellucci MEM, Canteras NS, Cunha Cda. Comparison of bilaterally 6-OHDA- and MPTP-lesioned rats as models of the early phase of Parkinson's disease: histological, neurochemical, motor and memory alterationsJ Neurosci Methods 2005;148:78-87.

Frisina PG, Hroutunian V, Libow LS. The neuropathological basis for depression in Parkinson's disease**.** Parkinsonism Relat. Disord*.*2009; 15:p.144–148,.

Graeff FG. On serotonin and experimental anxiety. Psychopharmacology 2002;163:467–476.

Halliday G. An Evidence Base for Noradrenergic Deficits in Parkinson's Disease. Mov Disorders 2012; 27: 1589-1591.

Hui, Y.P., Wang, T., Han, L.N., Li, L.B., Sun, Y.N., Liu, J., Qiao, H.F., Zhang, Q.J. Anxiolytic effects of prelimbic 5-HT1A receptor activation in the hemiparkinsonian rat. Behav. Brain Res. 2014; 277: 211-220.

Jungnickel J, Kalve I, Reimers L, Nobre A, Wesemann M, Ratzka A., et al. Topology of intrastriatal dopaminergic grafts determines functional and emotional outcome in neurotoxin-lesioned rats. Behav. Brain Res. 2011; 216, 129-135.

Kano O, Ikeda K, Cridebring D, Takazawa T, Yoshii Y, Iwasaki Y. Neurobiology of Depression and Anxiety in Parkinson's Disease. Parkinson's Disease 2011, Article ID 143547: 1-5.

Kish SJ, Tong J, Hornykiewicz O, Rajput A, Chang LJ, Guttman M, Furukawa Y.Preferential loss of serotonin markers in caudate versus putamen in Parkinson's disease. Brain 2008;131:120–131.

Kulisevsky J, Pascual-Sedano B, Barbanoj M, Gironell A,Pagonabarraga J, Garcia-Sanchez C. Acute effects of immediate and controlled-release levodopa on mood in Parkinson's disease: a double-blind study. Mov Disord 2007;22:62-67.

Lauterbach EC, Freeman A, Vogel RL.Correlates of generalized anxiety and panic attacks in dystonia and Parkinson disease. Cogn Behav Neurol 2003;16:225-233.

Leentjens AF, Dujardin K, Marsh L, Martinez-Martin P, Richard IH, Starkstein SE. Symptomatology and markers of anxiety disorders in Parkinson's disease: a cross-sectional study. Mov Disorders 2011; 26: 484-492.

Lima MMS, Braga Reksidler A, Marques Zanata S, Bueno Machado H, Tufik S, Vital MABF. Different parkinsonism models produce a time-dependent induction of COX-2 in the substantia nigra of rats. Brain Res 2006;1101:117-125.

Marinus J, Leentjens AF, Visser M, Stiggelbout AM, van Hilten JJ. Evaluation of the hospital anxiety and depression scale in patients with Parkinson's disease. Clin Neuropharmacol 2002; 25:318-324.

Martinez-Martin P, Damian J. Parkinson disease:depression and anxiety in Parkinson disease.Nature Reviews Neurology 2010; vol. 6, no. 5: 243–245.

Morais LH, Lima MMS, Martynhak BJ, Santiago R, Takahashi TT, Ariza D, Barbiero JK, Andreatini R, Vital MABF. Characterization of motor, depressive-like and neurochemical alterations induced by a short-term rotenone administration. Pharmacol. Rep.2012;64:1081-1090.

Negre-Pages L, Grandjean H, Lapeyre-Mestre M, et al. Anxious and depressive symptoms in Parkinson's disease: the French crosssectionnal DoPaMiPstudy. Mov Disord 2010;25:157-166.

Ninomiya EM, Martynhak BJ, Zanoveli JM, Correia D, Cunha Cda, Andreatini R.Spironolactone and low-dose dexamethasone enhance extinction of contextual fear conditioning. Prog Neuropsychopharmacol Biol Psychiatry 2010;34: 1229-35.

Nuti A, Ceravolo R, Piccinni A, Dell'Agnello G, Bellini G, Gambaccini G, et al. Psychiatric comorbidity in a population of Parkinson's disease patients. Eur J Neurol 2004;11:315-20.

Ozdilek B, Gunal DI. Motor and non-motor symptoms in Turkish patients with Parkinson's disease affecting family caregiver burden and quality of life. J Neuropsychiatry Clin Neurosci. 2012;24:478-483.

Pachana NA, Egan SJ, Laidlaw K, Dissanayaka N, Byrne GJ, Brockman S, et al. Clinical issues in the treatment of anxiety and depression in older adults with Parkinson's disease. Mov Disord 2013; 14:1930-4.

Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 5th edition. San Diego: Academic Press; 2005.

Pellow, S., Chopin, P., File, S.E. & Briley, M. Validation of open:closed arm entriesin an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 1985; 14, 149–167.

Picillo M, Amboni M, Erro R, Longo K, Vitale C, Moccia M et al. Gender differences in nonmotor symptoms in early, drug naive Parkinson's disease. J Neurol 2013; 260:2849-2855.

Pontone GM, Williams JR, Anderson KE, et al. Prevalence of anxietydisorders and anxiety subtypes in patients with Parkinson's disease. Mov Disord 2009;24:1333-1338.

Remy P, Doder M, Lees A, Turjanski N, Brooks D. Depression in Parkinson's disease: loss of dopamine and noradrenaline innervation in the limbic system. Brain 2005; 128:1314–1322.

Richard IH, Schiffer RB, Kurlan R. Anxiety and Parkinson's disease. J. Neuropsychiatry Clin. Neurosci. 1996; 8: 383-392.

Santiago RM, Barbiero J, Lima MMS, Drombrowski P, Andreatini R, Vital MABF. Depressive-like behaviors alterations induced by intranigral MPTP, 6-OHDA, LPS and rotenone models of Parkinson's disease are predominantly associated with serotonin and dopamine. Progress in Neuro-Psychopharmacology & Biological Psychiatry 2010; 34: 1104–1114.

Santiago RM, Barbiero J, Gradowski RW, Bochen S, Lima MMS, Cunha Cda, Andreatini R, Vital MABF. Induction of depressive-like behavior by intranigral 6-OHDA is directly correlated with deficits in striatal dopamine and hippocampal serotonin. Behavioural Brain Research 2014;259: 70– 77.

Sun Y-N, Wang T, Wang Y, Han L-N, Li L-B, Zhang Y-M, Liu J. Activation of 5-HT1A receptors in the medial subdivision of the central nucleus of the amygdala. Neuropharmacology 2015; 95:181-191.

Vázquez A, Jiménez-Jiménez FJ, García-Ruiz P, García-Urra D. "Panic attacks" in Parkinson's disease. A long-term complication of levodopa therapy. Acta Neurol. Scand. 1993:87:14-18.

Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nature Protocols 2007;2: 322-328. .

Walsh K, Bennett G. Parkinson's disease and anxiety. Postgrad Med 2001;77:89–93.

Wilson JM, Levey AI, Rajput A et al (1996) Differential changes in neurochemical markers of striatal dopamine nerve terminals in idiopathic
Parkinson's disease. Neurology 47:718–726.

CONCLUSÃO

Os modelos animais de DP utilizados nesse estudo apresentaram padrões diferentes de comportamento. Os dados referentes a administração bilateral e intranigral de 6-OHDA revelaram uma possível relação entre nível aumentado de 5-HT na amígdala e comportamento tipo ansioso. Porém, é necessário uma maior validação do modelo animal de 6-OHDA e testes para avaliação de ansiedade.

Já o tratamento por 10 dias consecutivos com uma baixa dose de rotenona via i.p. não resultou em difenças no comportamento relacionado a ansiedade, assim como não se alterou a concentração de serotonina na amígdala. Isso mostrou uma influência do comportamento com relação a interação desse neurotransmissor em relação as outras monoaminas em áreas límbicas e comportamento de ansiedade em DP.

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REFERÊNCIAS

AKIMOVA, E.; LANZENBERGER,R.; KASPER, S. The Serotonin-1A Receptor in Anxiety Disorders. **Biol psychiatry**; v.66, p.627–635, 2009.

ALAM, M., SCHMIDT, W.J. L-DOPA reverses the hypokinetic behaviour and rigidity in rotenone-treated rats. Behav. Brain Res. 153, 439–446, 2004.

BARA-JIMENEZ, W., *et al*. Effects of Serotonin 5-HT1A Agonist in Advanced Parkinson's Disease. **Movement Disorders**, v. 20, n. 8, p. 932–936, 2005.

BARONE, P. Neurotransmission in Parkinson's disease: beyond dopamine. **European Journal of Neurology**, 17: 364–376, 2010.

BEAL, M.F. Experimental Models of Parkinson's Disease. **Nature Reviews**, v.2, p. 325-332, 2001.

BEAR, M. F. *et al*. **Neurociências, desvendando o sistema nervoso.** Ed. Artmed, 3ª ed., 2008.

BLIER, P. Altered Function of the Serotonin 1A Autoreceptor and the Antidepressant Response. **Neuron,** 65, January 14, 2010.

BOGDANOVA Y, CRONIN-GOLOMB A. Neurocognitive correlates of apathy and anxiety in Parkinson's disease. **Parkinsons Disease**: 1-9, 2012.

Bond, A.J., *et al.* Buspirone decreases physiological reactivity to unconditioned and conditioned aversive stimuli. **Psychopharmacology** 165, 291e295, 2003.

Bonifati, V., et al. Buspirone in levodopa-induced dyskinesias. **Clin. Neuropharmacol**. 1, 73e82, 1994.

BOVÉ, J., PERIER, C. Neurotoxin-based models of Parkinson´s disease. **Neuroscience** 211: 51–76, 2012.

BRANCHI, I. *et al.* Nonmotor Symptoms in Parkinson's Disease: Investigating Early-Phase Onset of Behavioral Dysfunction in the 6-OHDA Rat Model. **Journal of Neuroscience Research**,n.86, 2008.

BRAAK, H., *et al.* Stages in the development of Parkinson's disease-related pathology. **Cell and tissue research**, v. 318, issue 1, p. 121-134, 2004.

CANNON, J.R., *et al.* A highly reproducible rotenone model of Parkinson's disease. **Neurobiology of Disease**, 34, p.279–290, 2009.

CAVINESS, J. N. Pathophysiology of Parkinson's disease behavior – a view from the network. **Parkinsonism and Related Disorders**, 20S1, p.39–43, 2014.

CELADA, P.; BORTOLOZZI, A.;ARTIGAS, F. Serotonin 5-HT1A Receptors as Targets for Agents to Treat Psychiatric Disorders: Rationale and Current Status of Research. **CNS Drugs,** Jun., 2013**.**

DAUER, W.; PRZEDBORSKI, S. Parkinson's Disease: Mechanisms and Models. **Neuron**., v. 39, p. 889-909, 2003.

DELAVILLE, C.; DEURWAERDÈRE, P.; BENAZZOUZ,A. Noradrenaline and Parkinson's Disease. **Frontiers in systems neuroscience**. Vol. 5, art. 31, 2011.

DESPLATS, P., *et al*. Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. **Proc Natl Acad Sci** 106:13010–13015, 2009.

DISSANAYAKA, N.N.N.W., *et al*. The Clinical Spectrum of Anxiety in Parkinson's Disease. **Movement Disorders**, Vol. 29, No. 8, p.967-975, 2014.

DISSANAYAKA, N.N.W., *et al.* Anxiety Disorders in Parkinson's Disease: Prevalence and Risk Factors. **Movement Disorders**, v. 25, n. 7, p. 838–845, 2010.

DUNNET, S.B.; LINDGREN, H.S. Cognitive dysfunction and depression in Parkinson's disease: what can be learned from rodent models? **European Journal of Neuroscience**, v. 35, p. 1894–1907, 2012.

FUCHS, F. D.; WANNMACHER, L.; FERREIRA, M. B. C. **Farmacologia clinica fundamentos da terapêutica racional**. 3. ed. Rio de Janeiro: Guanabara Koogan, 2004.

GRAEFF, F. G.; ZANGROSSI , H. Jr. The Dual Role of Serotonin in Defense and the Mode of Action of Antidepressants on Generalized Anxiety and Panic Disorders. **Central Nervous System Agents in Medicinal Chemistry**, v.10, n. 3, 2010.

HAWKES, C.H., DEL TREDICI, K., BRAAK, H. Parkinson's disease: a dualhit hypothesis. **Neuropathol Appl Neurobiol**.,33:599-614,2007.

HANSEN, C., *et al*. alpha-Synuclein propagates from mouse brain to grafted dopaminergic neuron and seeds aggregation in cultured human cells. **J Clin Invest** 121:715–725, 2011.

HERNÁNDEZ-MONTIEL, H. L. Aspectos moleculares y prospectos de terapias en la enfermedad de Parkinson. **Bioquímica** 31 (4): 146-158, 2006.

HOLMQVIST, S. *et al.* Direct evidence of Parkinson pathology spread from the gastrointestinal tract to the brain in rats. **Acta Neuropathol**., DOI 10.1007/s00401-014-1343-6; 16 pag. 9 OCT 2014.

JAUNARAJS, K.L.E., *et al*. Potential mechanisms underlying anxiety and depression in Parkinson's disease:Consequences of l-DOPA treatment. **Neuroscience and Biobehavioral Reviews**, v. 35, p.556–564, 2011.

KIM, D., *et al.* What is the clinical significance of cerebrospinal fluid biomarkers in Parkinson's Disease? Is the significance diagnostic or prognostic? **Exp. Neurobiol**. Dec 23(4):p.352-364, 2014.

LEE, H.J., *et al*. Dopamine promotes formation and secretion of non fibrilar alpha-synuclein oligomers. **Exp Mol Med**, 43:216-222, 2011.

LEE, F. J. S., LIU, F. **Genetic factors involved in the pathogenesis of Parkinson's disease**. Brain Research Reviews, 2008.

LIMA, M.M.S., *et al*. Different parkinsonism models produce a time-dependent induction of COX-2 in the substantia nigra of rats. **Brain Res**. 1101, p.117-125, 2006.

MEREDITH G. E.; SONSALLA P; CHESSELET M.F. Animal Models of Parkinson's Disease Progression. **Acta Neuropathol**. , v.115, n.4, p. 385–398, 2008.

MOREIRA, C.G. *et al.* Behavioral, Neurochemical and Histological Alterations Promoted by Bilateral Intranigral Rotenone Administration: A New Approach for an Old Neurotoxin. **Neurotox Res**., v.21, p. 291–301, 2012.

NICHOLSON, S.L; BROTCHIE, J.M. 5-hydroxytryptamine (5-HT, serotonin) and Parkinson's disease – opportunities for novel therapeutics to reduce the problems of levodopa therapy. **European Journal of Neurology**, v.9, Issue Supplement s3, p. 1–6, 2002.

NINOMIYA, E.M. *et al*. Spironolactone and low-dose dexamethasone enhance extinction of contextual fear conditioning. **Prog Neuropsychopharmacol Biol Psychiatry**,v.34, n.7, p. 1229-35, 2010.

PHANI,S; LOIKE,J.D.; PRZEDBORSKI, S. Neurodegeneration and Inflammation in Parkinson's disease. **Parkinsonism and Related Disorders**, v. 18S1, p. 207–209, 2012.

PICILLO, M. *et al*. Gender differences in nonmotor symptoms in early, drug naive Parkinson's disease. **J Neurol**; 260:2849-2855, 2013.

PIENAAR,I.S.; LU, B.; SCHALLERT, T. Closing the gap between clinic and cage: Sensori-motor and cognitive behavioural testing regimens in neurotoxin-induced animal models of Parkinson's disease. **Neuroscience and Biobehavioral Reviews**, v.36, p.2305–2324, 2012.

PIEPER, H.C. *et al.* Different methylation of the TNF-alpha promoter in cortex and substantia nigra: Implications for selective neuronal vulnerability. **Neurobiology of Disease,** v.32, issue 3, p. 521-527, 2008.

PREDIGER, R.D.S. *et al*. Anxiety in Parkinson's disease: A critical review of experimental and clinical studies. **Neuropharmacology**, v. 62, p. 115 - 124, 2012.

PRZEDBORSKI, S. Pathogenesis of nigral cell death in Parkinson's disease. **Parkinsonism & Related Disorders**, v. 11, Supplement 1, p. S3–S7, 2005.

REALE, M. *et al*. Peripheral cytokines profile in Parkinson's disease. **Brain, Behavior and Immunity**, v.23, p. 55-63, 2009.

REICHMANN, H., *et al*. Non-motor features of Parkinson's disease: depression and dementia. **Parkinsonism Relat. Disord**.; 15(3): 87-92, 2009.

SANTIAGO, R.M. **Estudo da Depressão Associada a Modelos Animais da Doença de Parkinson.** Dissertação (Mestrado em Farmacologia), Departamento de Farmacologia, Universidade Federal do Paraná. Curitiba, 2010.

SCHNEIER, F.R., *et al.* Low dopamine D(2) receptor binding potential in social phobia. **Am. J. Psychiatry** 157, 457e459, 2000.

SHERER, TB, *et al*. The rotenone model of Parkinson's disease in vivo: selective striatal oxidative damage and caspase-3 activation in nigrostriatal neurons. **Society for Neuroscience Abstract** No. 653.2, 2002.

STEIN, D.J., *et al*. Social anxiety disorder and generalized anxiety disorder: serotonergic and dopaminergic neurocircuitry. **J. Clin. Psychiatry** 63, S12eS19, 2002.

TABACH, R. Modelos Animais para o Estudo de Drogas Ansiolíticas. Pág. 159-181. In: CARLINI, E.A.; MENDES, F. R. (org.), **Protocolos em Psicofarmacologia Comportamental: um Guia para a Pesquisa de Drogas com Ação sobre o SNC, com Ênfase nas Plantas Medicinais.** São Paulo: Editora Fap-Unifesp, 2011.

TADAIESKY, *et al*. Effects of SR141716A on Cognitive and Depression-Related Behavior in an Animal Model of Premotor Parkinson's Disease. **Parkinson's Disease**, v. 2010, 6 p., 2010.

TAYLOR, T.N. Nonmotor Symptoms of Parkinson's Disease Revealed in an Animal Model with Reduced Monoamine Storage Capacity.**The Journal of Neuroscience**, v. 29, n.25, p. 8103– 8113, 2009.

TEIXEIRA, R.C.; ZANGROSSI, H. jr.; GRAEFF, F.G. Behavioral effects of acute and chronic imipramine in the elevated T-maze model of anxiety. **Pharmacol Biochem Behav.** , v.65, n.4, p.571-576, 2000.

WARRAICH, S. T.; ALLBUTT, H. N., BINLLING, R., RADFORD, J., COSTER, M. J., KASSIOU, M. HENDRESON, J. M. **Evaluation of behavioural effects os selective NMDA NR1A/2B receptor antagonist in the unilateral 6-OHDa lesion rat model**. Brain research Bulletin, 78:85-90, 2009.

ZANGROSSI, H. Jr., *et al*. Serotonergic regulation of inhibitory avoidance and one-way escape in the rat in elevated T- maze. **Neuroscience and Behavioral Reviews**, v.25, p.637-645, 2001.

ZANOVELI, J.M., *et al*. Facilitation of 5-HT1A-mediated neurotransmission in dorsal periaqueductal grey matter accounts for the panicolytic-like effect of chronic fluoxetine. **Int J Neuropsychopharmacol**., v.13, n.8, p.1079-1088, 2010.

ZANOVELI, J.M.; NOGUEIRA, R.L.;ZANGROSSI, H. Jr. Enhanced reactivity of 5-HT1A receptors in the rat dorsal periaqueductal gray matter after chronic treatment with fluoxetine and sertraline: evidence from the elevated T-maze. **Neuropharmacology, v.** 52, n.4, p.1188-1195, 2007.